Evaluation of Perennial Ryegrass Cultivars for Dairy Production under Different Pasture Management

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The main objective of this thesis was to evaluate perennial ryegrass (*Lolium perenne* L.) cultivars for dairy production under different pasture management. Five experiments were conducted in Canterbury, New Zealand to evaluate perennial ryegrass in terms of morphology (experiment 1), nutritive value (experiments 1 and 2), rumen degradation (experiment 3), dietary preference (experiment 4) and milk production (experiment 5). Pasture management included growing perennial ryegrass cultivars with and without white clover (*Trifolium repens* L.) (experiments 1, 2, 3 and 4) and defoliating at different times of day (experiments 2 and 5) during pasture regrowth (experiment 2).

In experiment 1, morphology and nutritive value of eight perennial ryegrass cultivars (AberMagic, Alto, Base, Bealey, Commando, Kamo, One50 and Prospect) growing with and without white clover were evaluated in a split-plot design at three phenological stages (pre-heading vegetative, reproductive and post-flowering vegetative). Pastures were managed under dairy grazing with irrigation and the annual nitrogen (N) fertiliser application rate was 325 kg N/ha. Compared with diploid cultivars, the tetraploid cultivars, Base and Bealey, had less dead material proportion (16.0% vs. 18.0% at the pre-heading vegetative stage; 22.0% vs. 27.0% at post-flowering vegetative stage) and greater lamina proportion (51.7% vs. 45.1% at the pre-heading vegetative stage; 55.0% vs. 50.7% at post-flowering vegetative stage) at the vegetative stages. The high-sugar diploid cultivar, AberMagic, had the greatest water-soluble carbohydrates (WSC) concentration at all stages in both lamina and pseudostem. Organic matter digestibility in dry matter (DOMD) was greater in Base, Bealey and AberMagic (mean = 75.9%) than other cultivars (mean = 73.8%). However, the differences in morphology was not the root cause of the variation in chemical composition and digestibility among cultivars. White clover had little effect on morphology of perennial ryegrass but significantly increased the crude protein (CP) concentration of accompanying perennial ryegrass at the pre-heading vegetative stage from 162 to 170 g/kg dry matter (DM). Interactions between perennial

ryegrass cultivar and the presence of white clover were found for herbage WSC, acid detergent fibre (ADF) and neutral detergent fibre (NDF) concentrations and DOMD at the reproductive stage only.

In experiment 2, herbage nutritive value variation of three perennial ryegrass cultivars (AberMagic, Bealey and Prospect) harvested at different times of day (from sunrise to sunset) during pasture regrowth (from 7 to 28 days after defoliation) was evaluated in monocultures and mixtures with white clover. AberMagic had a greater WSC concentration (162 g/kg DM) than Bealey (144 g/kg DM) and Prospect (112 g/kg DM). White clover proportions in perennial ryegrass swards (4 cm above ground level) increased during pasture regrowth from 6.5% on day 7 to 13.8% on day 28. The presence of white clover led to a greater overall herbage CP concentration (260 vs. 286 g/kg DM) and a lower NDF concentration (421 vs. 397 g/kg DM) in all cultivars. Herbage WSC concentration increased from 87 to 186 g/kg DM during the 4-week regrowth and was greater in the herbage harvested at sunset (180 g/kg DM) than sunrise (93 g/kg DM). In contrast, herbage CP concentration decreased during pasture regrowth from 292 g/kg DM to 242 g/kg DM and was lower in the herbage harvested at sunset (263 g/kg DM) than sunrise (293 g/kg DM). Herbage NDF concentration increased during pasture regrowth from 394 g/kg DM to 436 g/kg DM but dropped during the day from 439 g/kg DM to 378 g/kg DM. DOMD showed an opposite pattern to the NDF concentration, decreasing during pasture regrowth from 81.4% to 75.1% and increasing during the day from 76.4% to 81.2%. The interaction between perennial ryegrass cultivar and growth stage indicated that the cultivar ranking by CP and NDF concentration and DOMD changed during pasture regrowth and the differences in the WSC concentration among perennial ryegrass cultivars increased as regrowth proceeded.

In experiment 3, four rumen-fistulated Friesian × Jersey dairy cows were used in two Latin square designs to investigate *in sacco* rumen degradation characteristics of perennial ryegrass cultivars in monocultures and in mixtures with white clover at different proportions. Perennial ryegrass cultivars included AberMagic, Bealey and Prospect, and white clover proportions in the mixtures were 0%, 16.7%, 33.3% and 100% on a fresh weight basis. The tetraploid cultivar, Bealey, had a greater soluble fraction *a* and a faster insoluble-degradable fraction *b* of both DM (0.305 and 0.621/h, respectively) and organic matter (OM, 0.280 and 0.647/h, respectively). Regarding CP degradation, Prospect had a greater soluble fraction, *a* (0.132), than AberMagic (0.082) and Bealey (0.062). Bealey had the lowest accumulative and instantaneous N release rate relative to OM disappearance, indicating it had the best rumen N/energy balance and synchrony among cultivars. As white clover had a greater effective degradability of CP (76.6% vs. 60.5%) and effective rumen degradable protein (ERDP) concentration than perennial ryegrass (237 vs. 118 g/kg DM), the effective degradability and ERDP concentration of the mixtures increased proportionally as white clover proportion increased. Consequently, white clover exerted a negative effect on rumen N/energy balance and synchrony. However, the impact was limited when the white clover proportion was low (16.7% on a fresh weight basis). In the mixtures, white clover interacted with perennial ryegrass for DM and OM predicted potential rumen degradability but did not the effective degradabilities.

In experiment 4, dietary preference and selection of dairy cows for eight perennial ryegrass cultivars (AberMagic, Alto, Base, Bealey, Commando, Kamo, One50 and Prospect) growing with and without white clover was examined in a split-plot design at three phenological stages (pre-heading vegetative, reproductive and post-flowering vegetative). The dietary preference was defined as the decreasing rate of sward surface height at the beginning of grazing with minimum environmental constrains. The selection index was calculated based on pre-grazing herbage mass availability and post-grazing herbage mass residual of each cultivar. Results showed that the tetraploid perennial ryegrass cultivars, Base and Bealey, and the high-sugar diploid cultivar, AberMagic, were most preferred and selected by dairy cows. The proportion of perennial ryegrass, herbage WSC concentration and DOMD were positively correlated with dietary preference and selection indices, while negative correlations were found with the proportion of dead material and herbage ADF and NDF concentration. Although white clover had limited effect on sward structure and morphology of perennial ryegrass pastures, its presence (4.9% to 6.8% on a DM basis above ground level) increased overall herbage CP concentration (154 vs. 171 g/kg DM at pre-heading vegetative stage; 230 vs. 249 g/kg DM at post-flowering vegetative stage). The interaction between the presence of white clover and perennial ryegrass cultivar for dietary preference index indicated that the presence of white clover reduced the differences in dietary preference for perennial ryegrass cultivars. However, it did not lead to a significant re-ranking of cultivars preference order.

In experiment 5, the effect of perennial ryegrass cultivar (AberMagic and Prospect) and the timing of herbage allocation (in the morning at 0830 h or afternoon at 1630h) on herbage nutritive value and milk production of mid-lactation dairy cows were examined in a randomised block design. Twelve groups of four Friesian × Jersey cows were allocated to three replicates of four treatments over 10 days. There were no significant differences in sward structure and morphological characteristics between cultivars, except Prospect having a lower average tiller mass (43.1 mg DM/tiller) than AberMagic (48.4 mg DM/tiller). The concentration of WSC and DOMD was greater in AberMagic (180 g/kg DM, 74.2%) than Prospect (153 g/kg DM, 71.4%). Herbage DM percentage, WSC concentration and DOMD were lower in the morning compared with in the afternoon (22.3% vs. 18.8%; 154 vs. 179 g/kg DM; 72.1% vs. 73.5%). Herbage DM intake (mean = 12.0 kg/cow/day), milk yield (mean = 17.2 kg/cow/day) and milksolids yield (mean = 1.60 kg/cow/day) did not differ significantly among treatments. Milk urea nitrogen (MUN) concentration decreased when Prospect was allocated in the afternoon compared with in the morning (8.39 vs. 9.33 mmol/L), while MUN concentration remained similar whether AberMagic was allocated in the morning or afternoon (8.95 vs. 8.96 mmol/L). This interaction suggested that the effects of allocation timing may potentially be different for different perennial ryegrass cultivars.

Pasture management exerted more pronounced and consistent effects than the effects of perennial ryegrass cultivar on herbage nutritive value. Interactions were found between perennial ryegrass cultivar and mangement for herbage nutritive value (the regrowth stage) and dietary preference (the presence of white clover). Therefore, pasture management could potentially be a more effective way to manipulate herbage nutritive value than perennial ryegrass cultivar. Additionally, the evaluations of perennial ryegrass cultivars should be conducted under a defined and consistent condition to exclude the management effects and cultivar × management interactions for herbage nutritive value. Further, in order to achieve a higher herbage feeding value, management could be adjusted to benefit a particular cultivar, and cultivars, in turn, could be selected to fit a certain management system better.

Keywords: perennial ryegrass, white clover, tetraploid, diploid, high-sugar cultivar, cultivar × management interaction, morphology, nutritive value, regrowth stage, diurnal variation, *in sacco* rumen degradation, nitrogen/energy balance and synchrony, dietary preference, dietary selection, herbage allocation time, nitrogen-use efficiency.

Dedication

To the memory of my beloved maternal grandmother

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List of Abbreviations

Acid detergent fibre	ADF
Adenosine triphosphate	ATP
Agricultural and Food Research Council	AFRC
Crude protein	CP
Cultivar selection differential	CSD
Dry matter	DM
Effective rumen degradable protein	ERDP
Forage Value Index	FVI
Fresh weight	FW
Livestock Improvement Corporation Limited	LIC
Milk urea nitrogen	MUN
National Forage Variety Trial	NFVT
Near infrared reflectance spectroscopy	NIRS
Neutral detergent fibre	NDF
New Zealand Plant Breeding and Research Association	NZPBRA
Nitrogen	N
Nitrogen-use efficiency	NUE
Non-structural carbohydrates	NSC
Organic matter	OM
Organic matter digestibility	OMD
Organic matter digestibility in dry matter	DOMD
Rising plate meter	RPM
Rumen degradable protein	RDP
Sward surface height	SSH
Water-soluble carbohydrates	WSC

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Chapter 1
Introduction

1.1 Background

The New Zealand dairy industry, including dairy cattle farming ($ 3.1 billion) and dairy product manufacturing ($ 3.7 billion) contributed to 2.84% of the Gross Domestic Product (GDP) in the year ended March 2015 (Statistics New Zealand 2015). In the season 2015/16, the total cow population was 5.00 million at an average stocking rate of 2.85 cows/ha (LIC & DairyNZ 2016). Across New Zealand, dairy farming occupies 1.75 million effective hectares out of a total of 10.7 million ha exotic grassland (Ministry for the Environment 2012; LIC & DairyNZ 2016). Dairy companies processed 20.9 billion L of milk, equivalent to 1.86 billion kg of milksolids in the season 2015/16 (LIC & DairyNZ 2016). Approximately 95% of dairy product of New Zealand is exported, and represents nearly one-third of the international dairy trade (Jay & Morad 2007).

The livestock industry in New Zealand is pasture based and relies on the control of calving dates to match animal demand with seasonal pasture supply (Woodfield & Easton 2004). This is essential to produce quality dairy product at competitive prices in the international market. Perennial ryegrass (*Lolium perenne* L.) is the most widely sown temperate grass species in New Zealand (Stewart et al. 2014). It is characterised by its outstanding ability to produce numerous tillers, as well as its regrowth potential following grazing, which ensures its high annual dry matter (DM) yield and grazing tolerance. The high crude protein (CP) concentration (averaged at 180 to 250 g/kg DM) and low fibre concentration (neutral detergent fibre (NDF), averaged at 400 to 500 g/kg DM) make perennial ryegrass palatable with a good nutritive value (Clark & Kanneganti 1998). Additionally, perennial ryegrass pasture is easy to establish and manage, and persists well under New Zealand's wide range of climates and weather conditions (Hunt & Easton 1989; Stewart et al. 2014).

Since perennial ryegrass was first introduced to New Zealand in early 19th century from Europe, new strains and cultivars have been developed to adapt to different environments and/or to meet various breeding objectives (Lee et al. 2012). Plant breeding conducted by competitive plant breeding companies leads to new forages and cultivars, and companies adjust their portfolio and implement market strategies based on their evaluations (Figure 1.1). Considering that 5% of New Zealand's dairy pasture is renewed each year, the evaluation and selection of cultivars is crucial for farmers (DairyNZ & NZPBRA 2012). DairyNZ developed the Forage Value Index (FVI), an independent, region, and season specific tool for perennial ryegrass cultivar selection (DairyNZ 2015; Chapman et al. 2016). The FVI is based on the National Forage Variety Trial (NFVT) system, which was set up in 1991 through New Zealand Plant Breeding and Research Association (NZPBRA) to cooperatively evaluate new breeding lines of perennial ryegrass by the plant breeding companies across a range of regions. Research has also evaluated perennial ryegrass cultivars to provide additional information besides herbage DM yield to breeders and farmers, such as nutritive value, preference, rumen degradation and animal performance (Tas et al. 2005; Smit et al. 2006; Tas et al. 2006a; Sun et al. 2010; Wims et al. 2013).

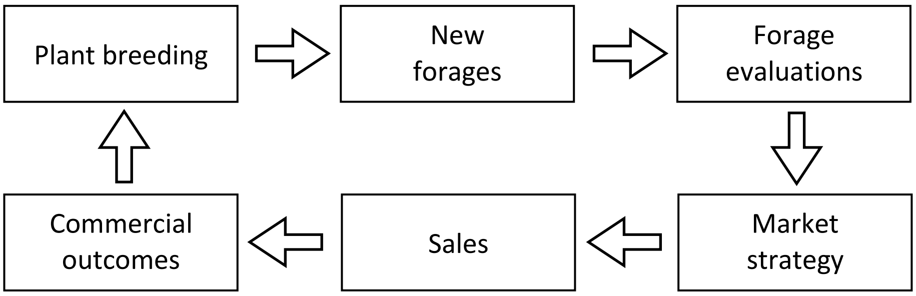


Figure 1.1 Forage improvement process (adapted from DairyNZ & NZPBRA, 2012).

Although the trials and evaluations conducted by plant breeding companies and researchers have been carried out using scientific protocols, the limitation is that the pasture management was standardized for the evaluations and may not reflect the conditions on a commercial farm. For example, cultivars were typically sown in evaluation as monocultures without white clover (*Trifolium repens* L.) or other species, while grass-legume mixtures are commonly used on a farm. Further, nitrogen (N) fertilisation rate, defoliation timing (regrowth intervals and times of day) and method (defoliation height) may also be different in an evaluation trial compared with a commercial farm. These pasture management options might exert more pronounced influences on perennial ryegrass cultivar evaluations than the cultivar *per se* and may interact with cultivars, and thus confound the results of the cultivar evaluations (Xie et al. 2014).

In previous studies, interactions have been reported between cultivar and pasture management, in terms of herbage DM yield (Cashman et al. 2016a), morphology (Hurley et al. 2009; Cashman et al. 2016b), chemical composition (Turner et al. 2015), rumen degradability (Sun et al. 2010) and dietary preference (Francis et al. 2006). For example, in mixed pastures, a small change in white clover proportion could lead to a disproportionate effect on animal production and outweigh the cultivar effect of perennial ryegrass (Hyslop et al. 2000). Likewise, a previous study (Gilliland & Mann 2000) showed that the lax and severe defoliation management could result in the re-ranking of perennial ryegrass cultivars in terms of annual DM yield.

1.2 Research objectives

The objective of this research was to investigate the effect of pasture management on evaluation of perennial ryegrass cultivars and interactions between them. Pasture management included growing perennial ryegrass with and without white clover, and defoliation at different regrowth stages and times of day. Factors related to herbage feeding value were evaluated, including morphology, chemical composition, rumen degradation, dairy cow dietary preference, DM intake and performance. The specific objectives were:

i. To investigate the differences in morphology and nutritive value of perennial ryegrass cultivars growing with and without white clover, and the relationship between morphology and nutritive value;

ii. To quantify the effects of perennial ryegrass cultivar, presence of white clover, regrowth stage and time of harvest during the day on pasture herbage nutritive value;

iii. To compare rumen degradation characteristics among perennial ryegrass cultivars with different proportions of white clover;

iv. To quantify dietary preference and selection of dairy cows for perennial ryegrass cultivars growing with and without white clover and to define what herbage characteristics contributed to dietary preference and selection;

v. To investigate the effect of perennial ryegrass cultivar, timing of herbage allocation and their interaction on herbage characteristics and milk production of mid-lactation cows.

1.3 Thesis structure

Thesis consists of eight chapters, as outlining in Figure 1.2. Chapter 1 is an introduction, and Chapter 2 is literature review that covers the key areas of perennial ryegrass cultivar evaluation and pasture management. Chapter 3 presents the evaluation of morphological characteristics and nutritive value of perennial ryegrass cultivars growing with and without white clover. Chapter 4 reports changes in perennial ryegrass herbage nutritive value at different times of day during pasture regrowth. Chapter 5 compares rumen degradation characteristics among perennial ryegrass cultivars with different white clover proportions. Chapter 6 demonstrates dietary preference and selection for perennial ryegrass cultivars growing with and without white clover. Chapter 7 reports animal performance of dairy cows grazing perennial ryegrass cultivars allocated at different times of day. Chapter 8 is a general discussion chapter, which draws together key conclusions. An overall conclusion, implications and further research suggestions are summarized at the end of the thesis.

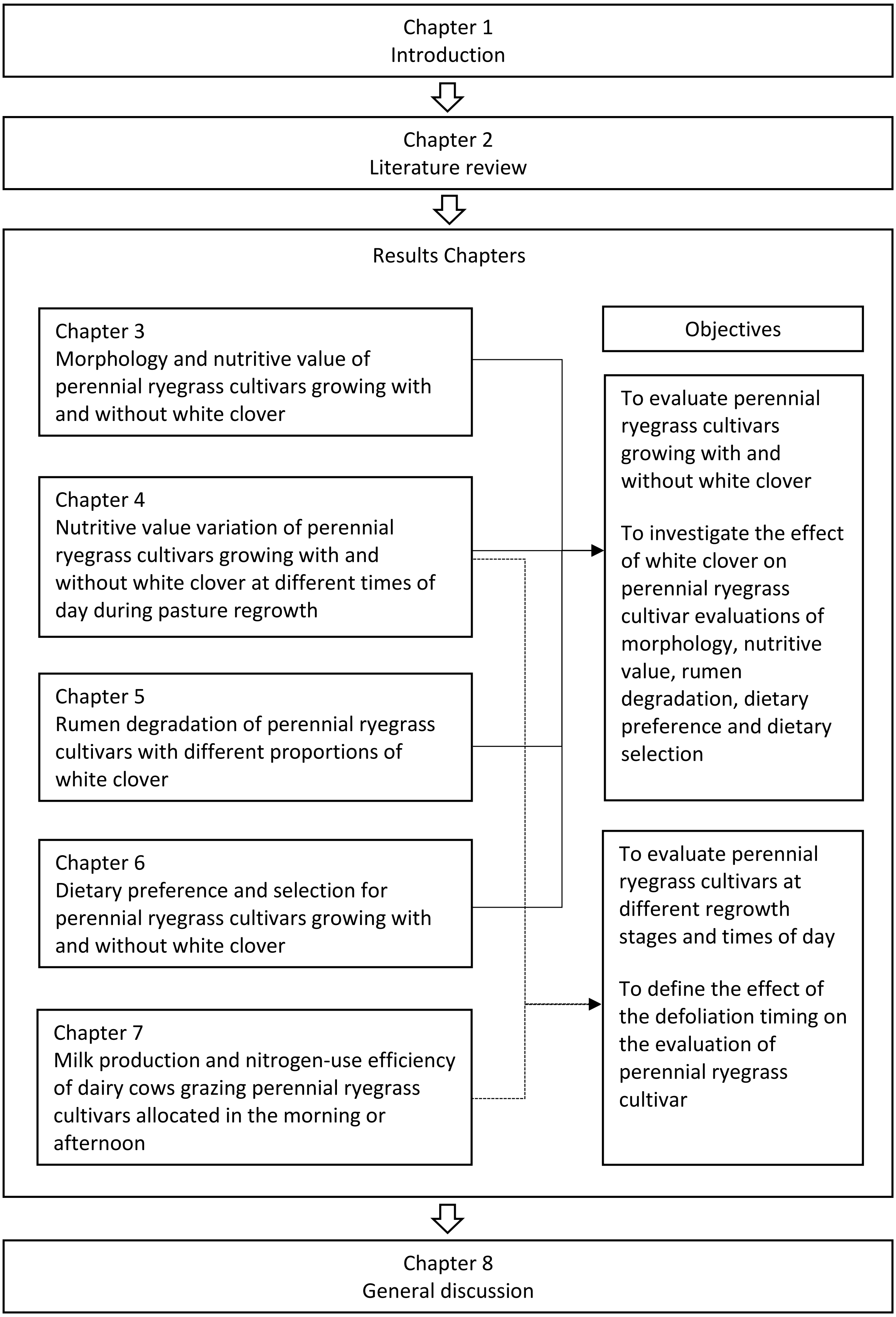


Figure 1.2 Diagrammatic representation of the thesis structure.

Chapter 2

Literature review

2.1 Perennial ryegrass breeding objectives

Perennial ryegrass (*Lolium perenne* L.) is the most widely sown temperate grass in north-west Europe, New Zealand, and the temperate regions of Japan, South Africa and South America (Humphreys et al. 2010). As a cross-pollinated species, its cultivars are populations of distinct, uniform, stable but genetically different due to the failure of self-pollination (Lee et al. 2012). Improvements in productivity and persistence so far have been achieved mainly by hybridisation and recurrent selection using the polycross method and by the use of polyploidy (Wilkins 1991). Also, molecular techniques are involved in more rapid and accurate breeding (Humphreys 2005). Previous perennial ryegrass breeding programmes have been mainly focussed on the following objectives: herbage dry matter (DM) yield, including annual DM yield and seasonal distribution (i.e. heading date), feeding value, resistance to diseases and pest, stress tolerance and persistence.

2.1.1 Herbage DM yield

Herbage annual DM yield

A major focus of perennial ryegrass breeding is to improve annual herbage DM yield. This is one of the key pieces of marketing information to seed companies and also customers, because of the strong link between herbage DM yield and farm profitability. The average rate of genetic gain in annual herbage DM yield was reported to be 0.76% per year or 105 kg DM/ha/year in New Zealand since 1990 (Harmer et al. 2016). Winter and early spring production may be worth disproportionately more than the spring and summer production, because of the early spring feed depletion (Stewart & Hayes 2011). According to the nationwide data from National Forage Variety Trial (NFVT) (Figure 2.1), the top-yielding cultivar in 2016 was Trojan. It yielded over 14,000 kg DM/ha per year, about 15% more than Nui, a standard cultivar developed in the 1970s (Armstrong 1977).

Herbage seasonal distribution

In addition to total herbage annual DM yield, the seasonal distribution of DM yield is also crucial. In New Zealand grazing systems, matching herbage DM yield with animal's requirements for feed throughout the year is important. Manipulating heading date is an effective way to achieve this. The time of heading determines the timing of early spring growth and has a significant effect on overall DM yield (Stewart & Hayes 2011). Heading date is defined as the date at which 50% of the tillers show ear emergence (Green et al. 1971). It can vary by up to 6 weeks among perennial ryegrass cultivars in spring, creating categories: early-, intermediate- and late-heading cultivars. Reproductive growth is highly related to the production and quality of perennial ryegrass (Minson 1960). In early spring, early-heading perennial ryegrass cultivars have a larger amount of herbage mass with lower quality, while late-heading cultivars produce herbage with a greater lamina proportion and a higher organic matter digestibility (OMD) (Laidlaw 2005; Humphreys & O'Kiely 2006). A reverse effect of heading date is found in summer, leading to similar annual herbage DM yield for perennial ryegrass cultivars with different heading date (Gilliland et al. 2002; Laidlaw 2004). Humphreys and O'Kiely (2006) found that the date of first-cut silage harvest of the late-heading perennial ryegrass cultivars could be delayed by 8 days achieving similar DM yield to the intermediate-heading cultivars or delayed by 30 days still achieving similar digestibility. After studying Holstein-Friesian cows for two successive years in Ireland, Gowen et al. (2003) reported that milk yield (22.4 vs. 21.3 kg/cow/ day), fat yield (0.86 vs. 0.82 kg/cow/day), protein yield (0.75 vs. 0.70 kg/cow/day) and lactose yield (1.04 vs. 0.98 kg cow/day) from late-heading perernnial ryegrass cultivars were significantly greater than the intermediate-heading counterparts.

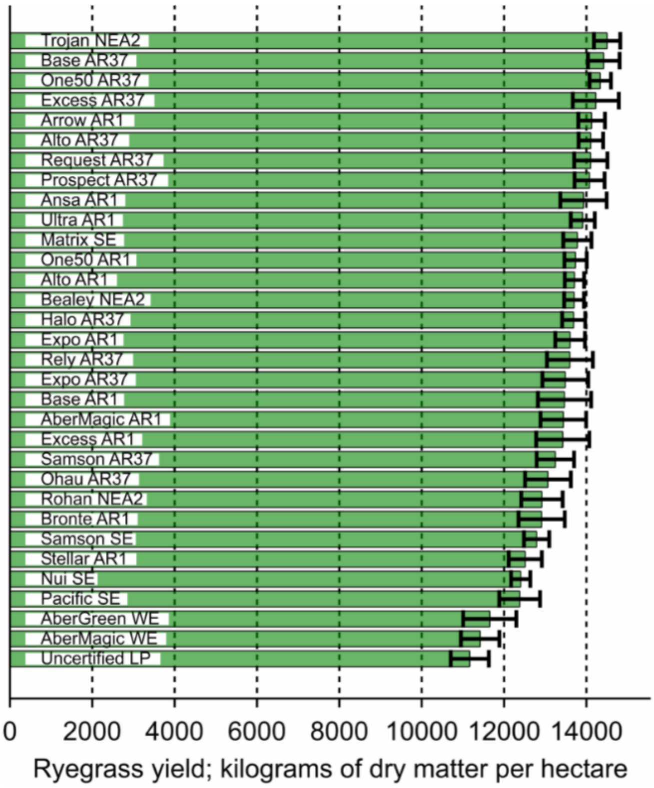


Figure 2.1 Annual herbage DM yield of perennial ryegrass cultivars in New Zealand (source: NZPBRA 2016). Cultivars whose error bars do not overlap are significantly different (*P* < 0.05). Uncertified LP is uncertified perennial ryegrass.

2.1.2 Herbage feeding value

Feeding value is a combination of herbage nutritive value and voluntary intake (Chapman et al. 2014). The ultimate objective of forage plant breeding is to improve animal performance. Animal performance is not only associated with pasture herbage DM yield, but also the ability to supply metabolisable nutrients and to maximize animal intake (Stewart & Hayes 2011). A range of breeding approaches have been developed to improve feeding value.

Aftermath heading

Perennial ryegrass reproductive growth may alter seasonal herbage DM yield distribution and reduce the quality (Laidlaw 2005; Humphreys & O'Kiely 2006). The reason is that perennial ryegrass produce reproductive stems that increase DM yield but reduce herbage quality during the reproductive stage (Chaves et al. 2006a). After the main flowering period in spring, aftermath heading usually occurs in summer, reducing the feeding value. Breeders have selected for reduced aftermath heading behaviour to enhance herbage digestibility and animal voluntary DM intake (Stewart & Hayes 2011; Fè et al. 2015).

Water-soluble carbohydrates

The concentration of WSC in perennial ryegrass varies from less than 50 g/kg DM to almost 400 g/kg DM, which could be affected by the climate and management. On average, the concentration of WSC is about 200 g/kg DM, and it mainly consists of fructan (around 70%), fructose, glucose and sucrose (McGrath 1988). It has been proved that a high WSC concentration is a consistent and heritable trait in perennial ryegrass breeding (Humphreys 1989a, b, c). Therefore, elevated WSC concentration perennial ryegrass cultivars, such as AberDart and AberMagic, have been developed, known as ‘high-sugar’ cultivars. Expression of the ‘high-sugar’ is associated with cool night temperatures (Parsons et al. 2004; Turner et al. 2015).

WSC might theoretically improve dairy production through two aspects. The first is to increase digestible DM intake by enhancing digestibility and DM intakes (Table 2.1). A greater WSC concentration is usually associated with lower ADF and NDF concentrations (Gregorini et al. 2006; Brito et al. 2009; Gregorini et al. 2009; Sauvé et al. 2009). Voluntary DM intake of forages by ruminants is largely affected by retention time in the rumen, which is principally depended on the fibre concentration in the diet (Thornton & Minson 1972). The second is to improve nitrogen-use efficiency (NUE) by providing rapidly fermentable carbohydrates and ultimately adenosine triphosphate (ATP) in the rumen (Hristov et al. 2005). Rumen degradable protein (RDP) and non-protein nitrogen (NPN) are broken down in the rumen and converted into ammonia and volatile fatty acid. If the energy supply is adequate, microorganisms in the rumen are able to capture more ammonia to produce microbial crude protein (MCP), thus potentially reducing the cost for expensive undegradable protein and increasing the proportion of nitrogen (N) excretion in faeces, a more stable form against N leaching compared with urine (Totty et al. 2013). If the energy supply is insufficient or asynchronic, rumen-degraded protein is absorbed as ammonia via the rumen wall, detoxified into urea in the liver and mostly excreted in urine (Tamminga 1996), resulting in low NUE and environmental pollutants, particularly as nitrate leaching and greenhouse gas emissions (Di & Cameron 2002). Microbes even use amino acids as an energy source when energy supply is severely deficient, thus leading to more ammonia accumulating in the rumen (Nocek & Russell 1988). Unfortunately, most forage sources are generally rich in RDP but deficient in non-structural carbohydrates (NSC) as an immediate energy supply (Kokko et al. 2013). Therefore, increasing the WSC concentration in perennial ryegrass as a rapidly fermentable energy source is considered to be a way to improve the NUE of dairy cows.

However, there were contradictory results of high-sugar cultivar evaluations in the literature. Miller et al. (2001) found that a high-sugar cultivar (Ba 11353) had a greater WSC concentration (165 vs. 126 g/kg DM) and a greater DM digestibility (71% vs. 64%). This resulted in a higher digestible DM intake (10.7 vs. 9.1 kg DM/day), even though the DM intakes were similar, which further led to greater milk yield (15.3 vs. 12.6 kg DM/day) of late-lactation dairy cows. However, Moorby et al. (2006) reported that DM intake (18.8 vs. 16.6 kg/day) and feed digestibility (75% vs. 72%) were greater in high-sugar perennial ryegrass cultivars, but there was no significant effect on milk yield. In a further study, Tas et al. (2006a) reported that cows grazing the cultivar with the lowest concentration of WSC had the lowest DM intake and milk yield in the first year of study, but with a similar difference in the WSC concentration (around 40 g/kg DM), dairy cow performance was not affected by perennial ryegrass cultivar in the second year of study. Moreover, Taweel et al. (2005a) and Taweel et al. (2005b) found that the significant differences in the WSC concentration among perennial ryegrass cultivars (181 vs. 153 g/kg DM; 125 vs. 111 g/kg DM; 181 vs. 157 g/kg DM; 180 vs. 149 g/kg DM) did not lead to significant differences in DM intake, rumen pH, rate of fibre degradation and milk yield in a stall-feeding experiment, and had no differences in grazing behaviour, DM intake, milk yield and milk composition in a grazing situation. Tavendale et al. (2006) failed to link the perennial ryegrass WSC concentrations to milk yield either. In terms of NUE, several studies observed a decrease in ruminal ammonia level (32.1 vs. 17.0 mg N/L in steers; 107.1 vs. 58.8 mg N/L in dairy cows) when dairy cows consumed perennial ryegrass cultivars with relatively higher WSC concentrations (Tavendale et al. 2006; Lee et al. 2012), thus improving NUE by reducing N excretion in urine (Miller et al. 2001; Moorby et al. 2006; Tas et al. 2006a). However, Tas et al. (2006b) found no effects of cultivars with different WSC concentrations on NUE of dairy cows.

Table 2.1 Digestibility (%) and DM intake (kg DM/day) of perennial ryegrass high-sugar and normal-sugar cultivars.

	Digestibility			DM intake		
	High-sugar	Normal-sugar	*P* value	High-sugar	Normal-sugar	*P* value
Miller et al., 2001	71	64	< 0.01	15.1	14.2	> 0.05
Moorby et al., 2006	75	72	< 0.05	18.8	16.6	< 0.05
Lee et al., 2002	61	56	< 0.05	9.3	6.7	< 0.05

Ploidy

Normal perennial ryegrass plants with two sets of chromosomes per cell are known as diploids, while tetraploids have four sets of chromosomes per cell. Due to the doubled chromosome number, tetraploid cultivars generally have larger cells with a greater cell content to cell wall ratio (Smith et al. 2001). Consequently, tetraploid cultivars produce larger and fewer tillers (90% heavier in tiller mass and 50% less in tiller density than diploid cultivars) and have a higher digestibility, crude protein (CP), and WSC concentrations (Smith et al. 2001). The open growth habitat of tetraploid perennial ryegrass cultivars with lower tiller density could provide more space and potentially support more legume than the dense sward of diploid cultivars (Frame & Boyd 1986). Additionally, lamina proportions were also reported to be greater in tetraploid cultivars than diploid cultivars (Gilliland et al. 2002; Orr et al. 2004) (Table 2.2). However, in monoculture situations, ploidy failed to exert any influences on intake rate, grazing time and animal performance under rotational grazing with cattle. Similarly, Gowen et al. (2003) found that there was no significant difference in animal performance when dairy cows were offered either diploid or tetraploid perennial ryegrass cultivars.

Table 2.2 Digestibility (%) and lamina proportion (%) of perennial ryegrass diploid and tetraploid cultivars.

	Digestibility			Lamina proportion		
	Diploid	Tetraploid	*P* value	Diploid	Tetraploid	*P* value
Gowen et al., 2003				60	62	< 0.05
				62	65	< 0.05
Laidlaw 2004				64	69	< 0.05
Solomon et al., 2014	68	72	< 0.05	25	28	< 0.05
	77	78	> 0.05	49	56	< 0.05
Balocchi et al., 2015	75	76	< 0.05			
	73	74	< 0.05			
	77	78	> 0.05			

2.1.3 Resistance and persistence

Cultivars should have adequate tolerance to stresses, such as winter cold stress, summer drought stress, intensive defoliation and tramping, and resistance to pests and diseases, such as Argentine stem weevil (*Listronotus bonariensis*) and crown rust (*Puccinia coronata*) (Stewart & Hayes 2011).

Tetraploid perennial ryegrass cultivars are generally less persistent than their diploid cultivars (Roegiers et al. 1988) because of their low tiller density (Wilkins & Humphreys 2003).

Endophytes (*Epichloë* spp.) protect against pest damage. They are fungi that invade the tissues of living plants for all or part of their life cycle and cause asymptomatic infections but no symptoms of the disease (Wilson 1995). Perennial ryegrass endophyte produces alkaloids, the plant secondary compounds, which could protect the plants from being damaged by pests. On the other hand, traditional endophyte strains may exert a negative influence upon grazing animal performance by causing ryegrass staggers and DM intake depression (Gallagher et al. 1981). However, novel endophyte strains, namely AR1, AR37, Endo5, Endosafe, NEA2 et al., which are developed by plant breeders to provide resistance to pests with reduced or no impacts on animal health and performance (Bluett et al. 2005; Johnson et al. 2013).

2.2 Evaluations of perennial ryegrass cultivars

2.2.1 Herbage DM yield

In New Zealand, the NFVT system was set up in 1991 through the NZPBRA, by the forage seeds companies to evaluate new breeding lines of perennial ryegrass. These trials have been conducted across a range of regions with replicated small plots for a three-year period, providing robust data on herbage DM yield and seasonal growth patterns as well as rust resistance (Easton et al. 2001; Kerr et al. 2012). Based on NFVT, DairyNZ developed the Forage Value Index (FVI), an independent, region and economically based evaluation index for perennial ryegrass in New Zealand dairy farm systems (DairyNZ 2015; Chapman et al. 2016). However, there are several limitations for economic cultivar evaluations. Firstly, feed quality is seldom included, except for digestibility in some occasions (McEvoy et al. 2011). Secondly, trials were typically conducted using pure perennial ryegrass swards with a high N fertiliser application rate, while often white clover (*Trifolium repens* L.) and other species were sown with perennial ryegrass in practice. Besides, all trials were conducted for three years, so the persistence of perennial ryegrass cultivars was not evaluated over longer time periods.

2.2.2 Herbage feeding value and animal performance

The ultimate goal of forage breeding is to achieve better animal performance. Therefore, in addition to herbage DM yield evaluation by plant breeding companies, feeding value and animal performance need to be evaluated among perennial ryegrass cultivars to provide breeders and farmers with useful information. Herbage feeding value includes herbage nutritive value and voluntary intake (Chapman et al. 2014) which is associated with sward structure, morphology, rumen degradation, dietary preference.

Herbage nutritive value depends on the concentration of nutrients and their availability to the animals. However, the concentration of nutrients varies in different seasons, at different regrowth stages and times of day (Fulkerson et al. 1998; Smith et al. 1999; Delagarde et al. 2000; Bryant et al. 2012), making it difficult to be monitored. Taking the high-sugar trait as an example, the expression of the higher WSC phenotype is depended on the environmental conditions (Francis et al. 2006). Cosgrove et al. (2007) reported that the high-sugar cultivars had 20-40 g/kg DM higher WSC concentrations in spring than the normal-sugar cultivar, whereas in autumn the differences were smaller and not significant. Smaller differences in the WSC concentrations among cultivars were also found at earlier regrowth stages compared with more mature stages (Turner et al. 2015).

Non-nutritional characteristics of vegetation, including sward structure and morphology, may exert an influence on grazing herbage DM intake (Hodgson 1985). DM intake is a function of bite rate, bite mass and grazing time (Laca et al. 1992). Bite rate is linked to the feed texture that is determined by botanical composition and morphology, while bite mass is related to bite depth and bulk density (Smit et al. 2005; Cosgrove & Edwards 2007). Cattle regulate the bite depth to remove a certain proportion (30 to 50%) of the sward surface height (Wade et al. 1989). Grazing animals are reluctant to penetrate into the lower layers containing pseudostems and dead material because of the lower digestibility and N concentration (Barthram & Grant 1984). Further, cultivars are significantly different in tiller density. Although a greater tiller density does not necessarily guarantee a higher herbage DM yield, tiller density is highly related to the bulk density, which is related to bite mass. Herbage intake rate of grazing animals increased as tiller density increased following the law of diminishing returns (Black & Kenney 1984). Also, morphology may affect dietary preference (Smit et al. 2006) and potentially herbage DM intake (Smit et al. 2005), because lamina has a higher nutritive value and is easier to harvest than stems and pseudostems (Chapman et al. 2012). As sheaths aggregate into pseudostems, a longer sheath length may result in a lower milk and milksolids yield because of the lower DM intake (Wims et al. 2013).

For rumen degradation, the ability to rapidly degrade in the rumen is a desirable trait for selection as it could lead to increased voluntary DM intake by grazing animals (Sun et al. 2010). After investigating 77 perennial ryegrass cultivars, Sun et al. (2010) suggested that cultivars were different in rumen degradation characteristics. The breeding criteria of DM degradation suggested by Sun et al. (2012) was a greater soluble fraction and a faster degradable fraction. ‘High-sugar’ cultivars are expected to provide rapidly fermentable carbohydrates for microorganisms in the rumen to capture more N into milk and excrete less into the urine (Miller et al. 2001).

An understanding of the dietary preference and selection of grazing animals is important for the efficient utilization of pasture (Rutter et al. 2004b). It is also useful for both farmers and breeders to improve animal voluntary DM intake and performance (Edwards et al. 2008). Dietary preference and selection by dairy cows, beef cattle and sheep have been evaluated for perennial ryegrass cultivars (Roegiers et al. 1988; Smit et al. 2006; Solomon et al. 2014). Previous studies (Mayland et al. 2000; Smit et al. 2006) showed that preference was positively correlated with herbage digestibility and the WSC concentration, and negatively with fibre concentrations. Tetraploid cultivars were characterised by a great WSC concentration and digestibility, and a low fibre concentration which may contribute to their better dietary preference (Roegiers et al. 1988).

2.3 Pasture management and the interaction with cultivar

2.3.1 White clover

Benefit of mixed sowing with white clover

White clover is a legume, characterised by its prostrate network of stolons, wide spread adventitious roots and dense leaves. It is widely used in pasture-based dairy production systems because of its N-fixation ability, superior nutritive value, seasonal herbage complementarity with grasses and milk yield increasing potential.

Most importantly, white clover is able to fix N through the association with microorganisms that form root nodules (Langer 1990). N fixation by white clover enables low cost, low input and high NUE dairy farming systems in temperate regions of the world (Frame et al. 1998; Ledgard et al. 1998). In a well-developed perennial ryegrass-white clover pasture, about 100-200 kg N/ha can be expected to be fixed from the atmosphere every year. Herbage DM yield and milk production from perennial ryegrass-white clover pasture are similar to those from a perennial ryegrass monoculture pasture receiving 200 kg N/ha fertiliser per year and about 70% of those from a perennial ryegrass monoculture with N fertiliser application of 350-400 kg N/ha/year (Andrews et al. 2007). N leaching in an unfertilised perennial ryegrass-white clover pasture was 30% to 50% relative to a perennial ryegrass monoculture receiving 200 kg N/ha/year (Laidlaw & Teuber 2001; Andrews et al. 2007). Therefore, reducing the reliance on N fertiliser by including white clover in perennial ryegrass pastures may reduce inorganic N input costs and risks of N leaching and greenhouse gas emissions resulting from N fertiliser production and application.

Forage legumes generally have higher concentrations of CP and minerals, and lower proportions of cellulose, hemicellulose and WSC, compared with perennial ryegrass (Andrews et al. 2007). As a result, adding legumes, such as white clover, alfalfa and vetch, to grass pastures increases the overall CP concentration of herbage (Zemenchik et al. 2002; Berdahl et al. 2004). The CP concentration of the grass fraction may increase as well by N transfer from legumes to grass via decay of plant tissues, root exudation of ammonium and amino acids and mycorrhizal hyphae (Paynel et al. 2008). Further, due to reticulum venations and a lower cell wall content, white clover and other legumes have faster rates of particle breakdown (Wilman et al. 1996) and greater rumen degradabilities than perennial ryegrass with parallel venation and a greater NDF concentration (Fulkerson et al. 1998) (Table 2.3).

In terms of herbage DM yield, different plant species show their maximum growth rates at different times of year (Holmes et al. 1987). In perennial ryegrass-white clover mixtures, white clover has a propensity to fill the gaps made available by the mid-summer depression of perennial ryegrass growth. This results in greater overall herbage DM yield (Evans et al. 1996), leaf area index and light interception abilities (Gerber & Gerber 2000). This increase in total herbage DM yield could be impaired by N fertiliser application. Chapman et al. (2016) found a greater advantage in herbage DM yield for perennial ryegrass-white clover mixtures under low N rates (mean = 3.08 t DM/ha/year under 42 and 101 kg N/ha/year) compared with high N rates (mean = 1.54 t DM/ha/year under 215 and 314 kg N/ha/year). Further, the difference may be fully compensated when the N application rate was as high as 500 kg N/ha/year (Reid & Strachan 1974).

In addition to herbage nutritive value and DM yield, milk production from perennial ryegrass-white clover mixtures was significantly enhanced compared with perennial ryegrass monocultures (Kunelius & Narasimhalu 1983; Berdahl et al. 2001; Wu et al. 2001; Zemenchik et al. 2002; Cosgrove 2006; Thompson 2013). Without N input, cows grazing a perennial ryegrass-white clover mixture produced more milk than those grazing perennial ryegrass only (20.4 vs. 19.8 kg/cow/day) (Phillips & James 1998). Including white clover in N-fertilized perennial ryegrass swards (250 kg N/ha/year) also increased milk production, compared with the perennial ryegrass monoculture (18.9 vs. 17.9 kg/cow/day), particularly in the second half of the lactation (Egan et al. 2017). In a study with sheep, even small increases in white clover proportion exerted large positive effects on liveweight gain from both perennial ryegrass and tall fescue (*Festuca arundinacea* L.) based pastures (Figure 2.2). However, the proportion of white clover may not remain at a high level in mixed-species pastures (Cosgrove et al. 2006). Especially in early spring and late autumn, when the white clover proportion is particularly low (below 20%), any 'superior' feeding value of white clover over perennial ryegrass may be diluted and hard to be reflected on milk production and quality (Andrews et al. 2007). The potential to improve milk production from a perennial ryegrass-white clover mixed pasture may be explained by the following reasons. The first is the better nutritive value, including the higher herbage CP concentration and lower fibre concentrations (Weller & Cooper 2001; Andrews et al. 2007; Allsop et al. 2009). The second is the greater rates of particle breakdown (Wilman et al. 1996) and degradation of white clover (Steg et al. 1994; Burke et al. 2000; Williams et al. 2005a; Williams et al. 2005b), leading to a greater DM intake and digestible DM intake. The third is that the energy cost for ingestion is lower for white clover than perennial ryegrass, resulting in more energy available for milk production at any given metabolisable energy intake (Nicol & Edwards 2011).

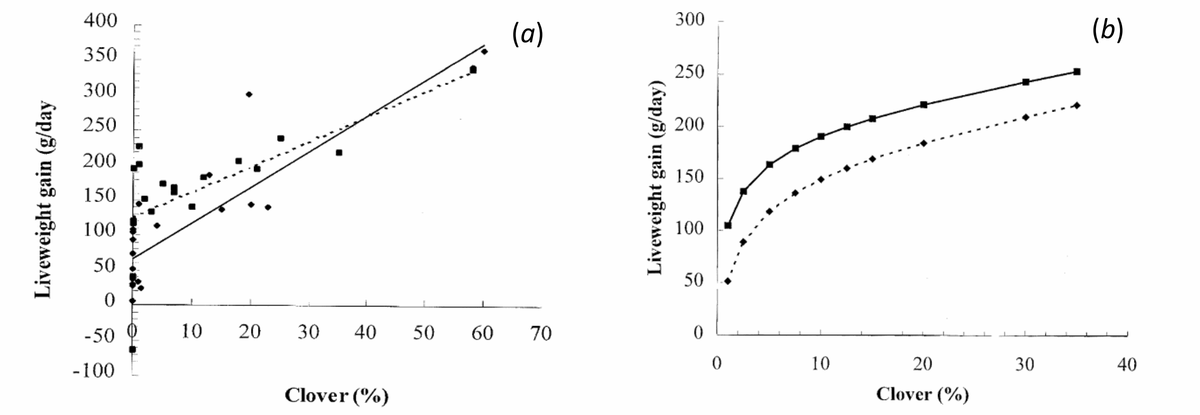


Figure 2.2 Spring liveweight gains (g/head/day) in young sheep grazing tall fescue (■) or perennial ryegrass (♦) swards of different white clover (*a*). Predicted relationship between percentage white clover in the diet on offer and liveweight gain in young sheep fed either tall fescue (■) or perennial ryegrass (♦) (*b*) (Hyslop et al. 2000).

Interaction between white clover and perennial ryegrass cultivar

In Canterbury, New Zealand, Rossi (2016) found that annual herbage DM yield of perennial ryegrass with white clover was 23% and 28% greater than that without white clover in two consecutive years, especially in the summer and autumn. However, no evidence of re-ranking in perennial ryegrass cultivars emerged and thus cultivar evaluations of annual herbage DM yield did not need adjustment to account for the effects of white clover (Easton et al. 2001; Rossi et al. 2014). In addition to herbage DM yield, feeding value is the other aspect of evaluation reflecting the ability to supply metabolisable nutrients and the ability to maximise animal intake (Stewart & Hayes 2011). It is related to herbage chemical composition, sward structure, morphology, rumen degradation and dietary preference (Laca et al. 1992; Smit et al. 2006; Edwards et al. 2008; Beecher et al. 2015). The potential interactions between white clover and perennial ryegrass cultivar for the herbage feeding value related factors could be due to the following reasons.

First, perennial ryegrass cultivars with an open growth habitat and a lower tiller density, such as tetraploid cultivars, could hypothetically support more white clover than those diploid cultivars with a dense sward (Frame & Boyd 1986). Perennial ryegrass cultivars may shade the white clover canopy, consequently reducing white clover growth and suppressing it competitively (Stern & Donald 1962). Therefore, perennial ryegrass cultivars with different sward structures and heights may potentially support a different amount of white clover and thus perform differently in cultivar evaluations when white clover is included. As mentioned above, Hyslop et al. (2000) reported that a small change in white clover content could lead to a disproportionate effect on animal production and outweigh the cultivar effect of perennial ryegrass.

Second, the presence of white clover in perennial ryegrass pastures increased soil N levels (Enriquez-Hidalgo et al. 2016) and perennial ryegrass cultivars would react differently to the N fertilisation. There were annual herbage N yields re-ranking at different N fertiliser application rates for perennial ryegrass cultivars (Elgersma et al. 2000). Bahmani et al. (2001b, 2001a) found interactions between cultivar and N application in tiller density and reproductive tiller development. The WSC concentration, CP concentration and herbage DM yield of perennial ryegrass cultivars also responded differently to N application in some occasions (Conaghan et al. 2012). As mentioned above, up to 100-200 kg N/ha can be fixed by white clover annually in a well-developed perennial ryegrass-white clover pasture. Therefore, perennial ryegrass cultivars could potentially respond differently to the presence of white clover due to the N fixation.

Third, because of the substrates in plant species, the digestive interaction of mixed diets might alter rumen degradability (Niderkorn et al. 2012). For example, condensed tannins, a plant secondary compound, are able to inhibit the proteolytic enzyme and reduce proteolytic bacterial populations in the rumen (Min et al. 2003; Burggraaf et al. 2008). Consequently, herbage protein rumen degradation may be suppressed (Niderkorn et al. 2012). Although white clover consists of tannin-free leaves, condensed tannins in the flower and other secondary compounds might potentially interact with perennial ryegrass cultivars in the rumen. Therefore, rumen degradation of perennial ryegrass-white clover mixtures might depend on perennial ryegrass cultivars and white clover proportion, resulting in misleading predictions of the mixtures from individual dietary components (Niderkorn et al. 2011).

Fourth, dairy cows may graze selectively to balance and synchronise the supply of nutrients (principally N and energy) to the rumen so as to optimise microbial protein synthesis (Kyriazakis 1996; Rutter 2006). When N-rich white clover is included in the sward, their grazing strategy might change according to the nutrient profile of perennial ryegrass cultivars. Besides, the dietary preference has been previously shown for white clover over perennial ryegrass (Rogers et al. 1982; Rogers et al. 1998; Rutter et al. 2004a, b), but cows are not able to select white clover only from a mixture sward. Cosgrove et al. (2002) found that dietary preference between perennial ryegrass with a high and low N concentration was reduced when white clover was offered as another choice. Therefore, dietary selection and preference for perennial ryegrass cultivars might change when growing with white clover, compared with those in their monocultures.

2.3.2 Regrowth stage

Effects of growing season on perennial ryegrass cultivar evaluation have been well documented in terms of herbage DM yield and herbage nutritive value (Lovett et al. 2006; Purcell et al. 2012). Fulkerson and Donaghy (2001) suggested perennial ryegrass is a ’3-leaf’ plant with the initiation of the fourth leaf and the senescence of the oldest leaf happening simultaneously. Defoliation before two leaves/tiller reduced regrowth rate and persistence while grazing pasture older than three leaves/tiller may not only lead to herbage waste but also the decrease in overall herbage quality because of senescent material (Fulkerson & Donaghy 2001). Herbage DM accumulated quadratically during pasture regrowth with a decreasing DM degradation rate (Chaves et al. 2006a). Meanwhile, the concentrations of CP and ash, and digestibility decreased, while concentrations of ADF and NDF increased during pasture regrowth (Delagarde et al. 2000; Chaves et al. 2006a).

Sun et al. (2010) suggested that age of regrowth could lead to larger differences than perennial ryegrass cultivar in chemical composition and rumen degradation characteristics. Potentially, regrowth interval could exert substantial influences on morphology and nutritive value, thus confounding the effect of perennial ryegrass cultivar (Tas et al. 2006b; Sun et al. 2010). However, little information is available about the interaction between cultivar and regrowth stage in perennial ryegrass evaluation. Previous studies have provided evidence that perennial ryegrass cultivars performed differently at different regrowth stages. For example, Turner et al. (2015) compared perennial ryegrass cultivars under two contrasting defoliation interval treatments to evaluate the influence of pasture management on high-sugar trait expression. Results suggested that defoliation at 3-leaf stage of regrowth, rather than 1.5-leaf stage, led to a greater difference in herbage WSC concentration between high-sugar and normal-sugar cultivars (Turner et al. 2015). This probably because WSC were mobilized and consumed for producing new plant tissues at early regrowth (Donaghy & Fulkerson 1998; Morvan-Bertrand et al. 2001) when energy supply from photosynthesis is inadequate. Consequently, this interaction led to smaller variations among cultivars at earlier regrowth stages with low overall WSC concentrations (Morvan-Bertrand et al. 2001).

2.3.3 Time of day

Herbage DM percentage and the concentration of WSC increase dramatically during the day, because of the moisture loss and net photosynthesis accumulation (Orr et al. 2001b). Accordingly, the concentrations of CP, ADF and NDF decrease due to the dilution (Delagarde et al. 2000). An improvement in herbage digestibility has been recorded in the afternoon as well (Table 2.4). However, diurnal fluctuations of herbage chemical composition could be different among perennial ryegrass cultivars. For example, Turner et al. (2002) suggested that fructan exohydrolases might be less active in high-sugar perennial ryegrass cultivars than the normal-sugar counterparts, particularly against large polymers. Therefore, under environmental conditions that favour hydrolysis (e.g. continuously low light or night time), high-sugar cultivars would retain a part of their polymeric fructan, because high-sugar cultivars are associated with increased accumulation of large fructan polymers (Gallagher et al. 2015), but normal-sugar perennial ryegrass cultivars may deplete them all (Turner et al. 2002). This mechanism could theoretically result in a greater difference in the WSC concentration between high-sugar and normal-sugar cultivars after the night in the morning than the afternoon. However, little information is available on the interaction between time of day and perennial ryegrass cultivar in terms of nutritive value. Humphreys (1989b) claimed that genetic differences between high-sugar perennial ryegrass cultivars and normal-sugar cultivars could remain stable without large interactions with environment. Nevertheless, the expressions of the high-sugar trait varied under different temperatures (Parsons et al. 2004), at different sites (Halling et al. 2005), in different seasons (Cosgrove et al. 2007) and during different growth stages (Turner et al. 2006), and the interaction with the time of day might be involved.

Under grazing conditions, ruminants show a regular daily grazing pattern with the longest and most intense grazing event taking place in the afternoon (including dusk) (Figure 2.3). This is due to herbage nutritive value, rumen function and fill, photoperiod and predator avoidance strategies (Gregorini 2012). Therefore, matching this grazing pattern with the better nutritive value of afternoon herbage by altering herbage allocation timing might enhance the performance of livestock (Orr et al. 2001b; Gregorini 2012). Besides, afternoon herbage contains more WSC and less CP, providing more rapidly fermentable carbohydrates (Hristov et al. 2005) and producing less ammonia in the rumen (Tamminga 1996). Therefore, time of day may also be used as a management strategy to improve microbial synthesis efficiency and NUE of dairy cows (Vibart et al. 2012).

However, similar DM intakes and milk production were found in the morning and afternoon allocation system previously (Table 2.5). This might be due to a shorter continuous grazing time limiting DM intake when fresh herbage was allocated in the afternoon, as cows were reluctant to graze at night (Kilgour & Dalton 1984).

Time of day might thus affect and interact with cultivar evaluation in terms of animal behaviour and milk production. However, little attention has been given to this.

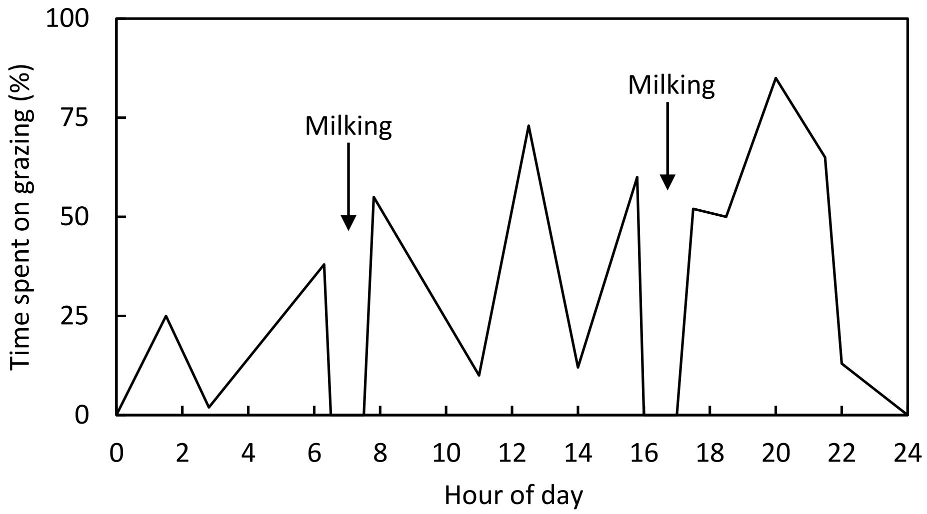


Figure 2.3 Changes in grazing intensity over the day for a lactating cow (adapted from Phillips 2002).

Table 2.3 Chemical composition (g/kg DM) and digestibility (%) of white clover and perennial ryegrass.

	WSC			CP			NDF			Digestibility		
	WC†	PRG§	*P* value	WC	PRG	*P* value	WC	PRG	*P* value	WC	PRG	*P* value
Hammond et al., 2011	98	114	-	255	192	-	276	444	-			
	125	164	-	214	125	-	269	415	-			
Francis et al., 2006	140	183	-	236	127	-	317	488	-	79	79	-
Rutter et al., 2002				329	189	-	226	548	-	77	60	-
Harris et al., 1998				215	93	-	376	613	-	75	67	-
				246	205	-	361	527	-	72	68	-
Orr et al., 1997	62	172	< 0.01	300	169	< 0.01				77	68	< 0.01
Allsop et al., 2009	117	220	> 0.05	294	154	< 0.05	182	431	< 0.01	72	73	> 0.05

†WC, white clover
§PRG, perennial ryegrass

Table 2.4 Chemical composition (g/kg DM) and digestibility (%) of perennial ryegrass pastures in the morning and afternoon.

	WSC			CP			NDF			Digestibility		
	AM†	PM§	*P* value	AM	PM	*P* value	AM	PM	*P* value	AM	PM	*P* value
Abrahamse et al., 2009	114	147	< 0.01	193	187	> 0.05	452	425	< 0.05	82	84	< 0.05
Vibart et al., 2012	140	157	< 0.10	176	180	> 0.05	505	499	> 0.05	82	83	> 0.05
Orr et al., 2001	175	204	< 0.05	219	206	> 0.05	423	420	> 0.05	73	73	> 0.05
Gregorini et al., 2006	103	163	< 0.05	144	129	> 0.05	460	400	< 0.05	72	75	< 0.05
Purwin et al., 2016	167	205	< 0.05	148	148	> 0.05	532	509	< 0.05	79	80	> 0.05

†AM, morning
§PM, afternoon

Table 2.5 DM intake (kg DM/cow/day) and milk yield (kg/cow/day) from perennial ryegrass pastures allocated in the morning and afternoon.

	DM intake			Milk yield		
	AM†	PM§	*P* value	AM	PM	*P* value
Abrahamse et al., 2009	16.3	15.4	> 0.05	26.3	26.0	> 0.05
Pulido et al., 2015	14.9	14.1	> 0.05	23.0	23.7	> 0.05
Orr et al., 2001	17.8	18.0	> 0.05	25.7	26.5	> 0.05

†AM, morning
§PM, afternoon

2.4 Summary

Perennial ryegrass cultivars have been bred for the improved herbage DM yield, feeding value, resistance and persistence. Evaluations of cultivars have been carried out by breeding companies and researchers using scientific protocols. However, in commercial farms, pasture management may be different. Pasture management, such as growing perennial ryegrass cultivars with and without white clover and defoliating at different timing, could potentially exert pronounced influences on perennial ryegrass cultivars and further interact with perennial ryegrass cultivar. However, there are knowledge gaps in the interactions between perennial ryegrass cultivar and pasture management in cultivar evaluations. This thesis focused on the evaluations of feeding value related factors of perennial ryegrass cultivars under different pasture management.

Chapter 3

Morphology and nutritive value of perennial ryegrass cultivars growing with and without white clover

3.1 Introduction

Perennial ryegrass (*Lolium perenne* L.) is one of the dominant grass species used in temperate regions of the world (Humphreys et al. 2010). In grazing pastures, perennial ryegrass is often grown with white clover (*Trifolium repens* L.) due to white clover's nitrogen (N) fixation and superior nutritive value, compared with perennial ryegrass (Evans et al. 1996; Chen et al. 2016). The grass-clover interactions in mixture pastures could potentially lead to perennial ryegrass cultivars benefiting from white clover at different extents (Chapman et al. 2018). However, previous studies have focused on the grass-clover interactions for herbage mass accumulation instead of plant morphology and nutritive value (Rossi 2016). As a legume, white clover could enhance soil N (Enriquez-Hidalgo et al. 2016) via decay of plant tissues, root exudation and mycorrhizal hyphae (Paynel et al. 2008). Meanwhile, Cookson et al. (2000) suggested that N input affected perennial ryegrass reproductive growth and morphology with an interaction with cultivars. On the other hand, perennial ryegrass cultivars differ in sward structures, which would potentially support different amounts of white clover in the mixtures (Stern & Donald 1962; Frame & Boyd 1986), thus affecting the overall herbage nutritive value (Chen et al. 2016). Consequently, the present of white clover in perennial ryegrass pastures would affect morphology and nutritive value of the accompanying perennial ryegrass and different perennial ryegrass cultivars may response differently.

Perennial ryegrass herbage consists of leaf, stem, inflorescence and attached dead material, and the leaf sheaths aggregate into pseudostems. Previous studies have shown that perennial ryegrass morphological components have different chemical compositions (Chaves et al. 2006b). Therefore, their proportions could potentially determine the herbage nutritive value of the entire plant. Especially in the reproductive stage, the elongation of stem and the development of inflorescence increase herbage dry matter (DM) yield at the expense of herbage nutritive value (Laidlaw 2005). Thus, it is critical to understand how herbage nutritive value varies in plant morphological components and whether it is affected by perennial ryegrass cultivar and pasture management.

Perennial ryegrass cultivars also differ in nutritive value. This may reflect the differences in morphology or alternatively the differences in chemical composition of the same morphological component among cultivars. Previous research has shown that tetraploid cultivars have a greater digestibility with a greater lamina proportion, compared with the diploid counterparts (Gilliland et al.

2002). Additionally, high-sugar cultivars generally have a greater proportion of pseudostem (Wims et al. 2013) while the majority of water-soluble carbohydrates (WSC) is stored in the leaf sheaths (Fulkerson & Donaghy 2001). Therefore, it is hypothesized that variations in nutritive value among perennial ryegrass cultivars may reflect the differences in their morphology. In this chapter, nutritive value of the entire plant and each morphological component were compared among perennial ryegrass cultivars.

The objectives of this study were (1) to evaluate the effect of white clover on morphology and nutritive value of accompanying perennial ryegrass and its interaction with perennial ryegrass cultivar and (2) to investigate the differences in morphology and nutritive value among perennial ryegrass cultivars and the relationship between perennial ryegrass morphology and its nutritive value.

3.2 Materials and methods

3.2.1 Experimental design

The experiment was conducted within an existing experiment (Rossi 2016) at Lincoln University Research Dairy Farm, Canterbury, New Zealand (43°38'S, 172°27'E, 12 m above sea level). Pastures were established in March 2012 in a split-plot design with five blocks. Twenty main-plots (18 m × 28.8 m) comprised all combinations of pastures sown with and without white clover receiving N fertiliser at 100 kg N/ha and 325 kg N/ha.

In this experiment, measurements were conducted in the first four blocks within the high N rate main-plots (Figure 3.1). Each main-plot contained eight perennial ryegrass cultivars in adjacent sub-plots (Table 3.1). Cultivars selected covered a range of ploidy (diploid and tetraploid), heading dates (from mid to late) and the WSC concentrations (high-sugar vs. normal-sugar). The sowing rates were based on seed size to generate similar plant populations at the establishment (Table 3.1). The white clover sown in the 'present' treatments was a 50:50 mixture of cultivar Kopu II and Tribute (sown at 4 kg/ha).

3.2.2 Management

All experimental areas were grazed by dairy cows at the same time for defoliation when the herbage mass was between 2500 and 3300 kg DM/ha from August to May the next year. The grazing intervals were 3 to 6 weeks depending on the season. The target post-grazing residual was 4 to 5 cm sward height (approximately 1500 – 1750 kg DM/ha). Typically, each main-plot was grazed by 10 to 11 cows over 6 to 8 hours depending on the herbage availability. N fertiliser was equally separated into ten applications after each defoliation at the rate of 32.5 kg N/ha after each defoliation. All experimental areas were irrigated by centre-pivot irrigation according to the schedule organized by the farm management team.

3.2.3 Sampling and processing

Herbage was sampled after 21, 32 and 26 days of regrowth at three phenological stages: pre-heading vegetative (early spring, October 2014), reproductive (late spring, November 2014) and post-flowering vegetative (autumn, May 2014), respectively. Herbage samples of approximately 150 g fresh weight were cut to ground level from at least six locations in each sub-plot. Fresh samples were immediately placed in a chilly bin and transferred to the laboratory as soon as possible. White clover was sorted from perennial ryegrass, and perennial ryegrass was separated into lamina, pseudostem and reproductive stem. Perennial ryegrass lamina was cut at its junction with the ligule and sheath. Pseudostems were defined as the accumulation of sheaths. Reproductive stems included true stems, attached sheaths and inflorescences. Dead material (any senesced material with yellow/brown colour) was separated from all the green materials (Figure 3.2a). All fractions were oven-dried at 60°C for 48 h and weighed (Figure 3.2b). The proportion of white clover and perennial ryegrass morphological components were calculated on a DM basis.

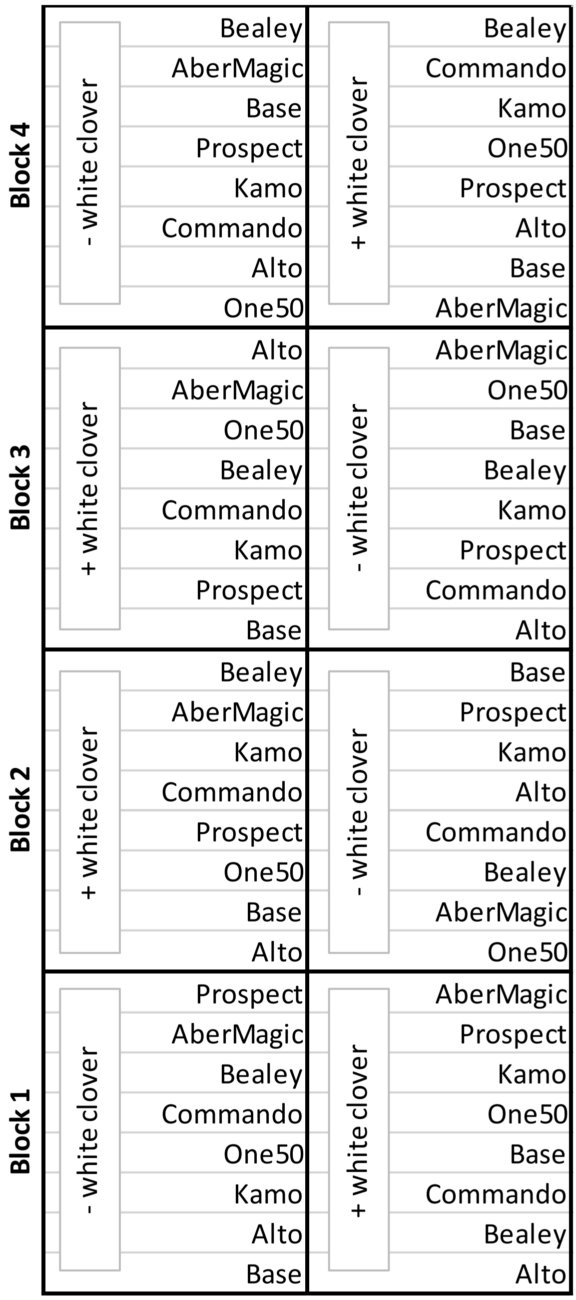


Figure 3.1 Experiment layout. '+ white clover' means white clover is present and '- white clover' means white clover is absent.

Table 3.1 Perennial ryegrass cultivars sown in the experiment.

Cultivars	Ploidy	Heading date† (d)	Endophyte	Seed rate (kg/ha)
Base	tetraploid	+22	AR37	28
Bealey	tetraploid	+25	NEA2	28
AberMagic§	diploid	+15	AR1	20
Alto	diploid	+14	AR37	20
Commando	diploid	+1	AR37	20
Kamo	diploid	0	AR37	20
One50	diploid	+20	AR37	20
Prospect	diploid	+12	AR37	20

†Days relative to cultivar Nui.
§High-sugar cultivar

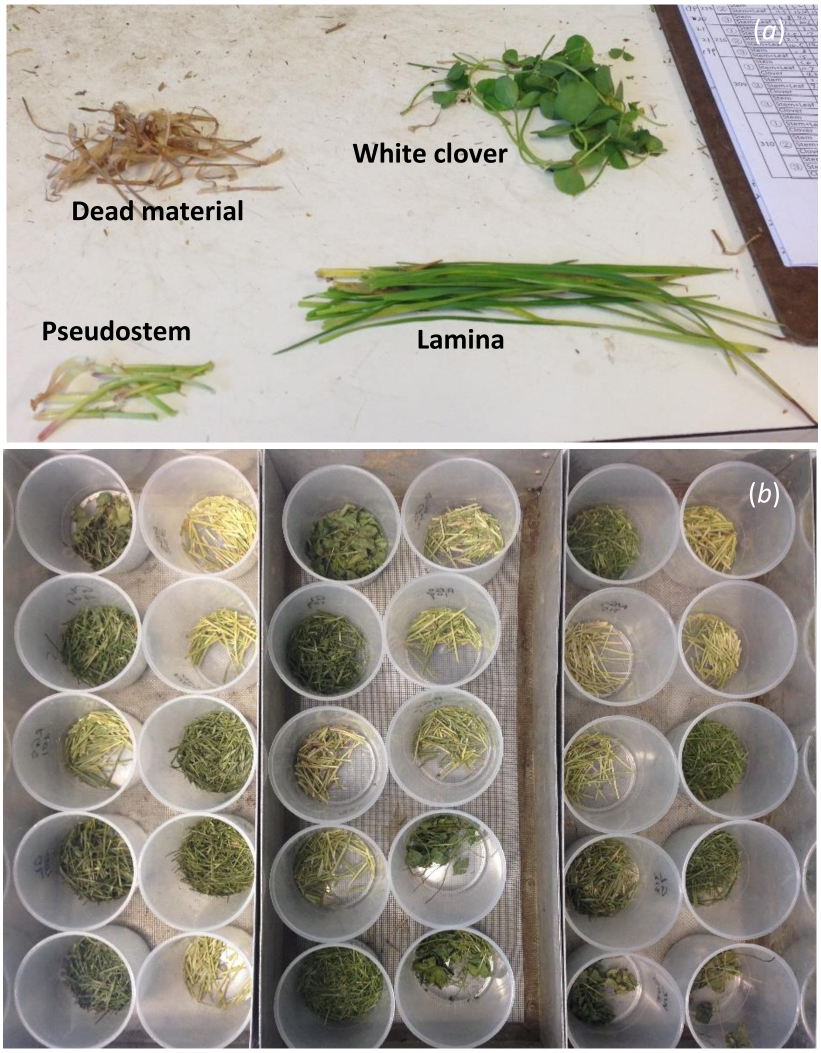


Figure 3.2 Sample processing (*a*) and oven-drying (*b*).

3.2.4 Chemical composition and digestibility analysis

The dead material was bulked from all treatments. This was because the proportion of dead material was too small to analyse separately for each treatment. All oven-dried fractions were ground through a 1-mm sieve individually. Ground samples were analysed by near infrared reflectance spectroscopy (NIRS Systems 5000, Foss, Maryland, USA) for chemical composition, including the concentrations of organic matter (OM), crude protein (CP), neutral detergent fibre (NDF), acid detergent fibre (ADF) and WSC, and organic matter digestibility in dry matter (DOMD). NIRS calibrations were previously derived on perennial ryegrass samples (Jones & Hayward 1975; MAFF 1986; AOAC 1990; van Soest et al. 1991). Assays for NDF excluded sodium sulphite or α-amylase and both ADF and NDF predictions were inclusive of residual ash (Bryant et al. 2012). The chemical composition and digestibility of the perennial ryegrass whole plant were calculated according to the proportion and nutritive value of each morphological component.

3.2.5 Statistical analysis

The effects of white clover (main-plot factor), perennial ryegrass cultivar (sub-plot factor) and their interactions on morphology, chemical composition and digestibility of perennial ryegrass entire plant were analysed by ANOVA (SPSS version 22) for each phenological stage using a split-plot model:

$$y = \mu + \gamma_k + \alpha_i + \delta_{ik} + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijk} ,$$

where y is the dependent variable, μ is the overall mean, α_i is the effect of white clover, γ_k is the block effect, δ_{ik} is the interaction between white clover and block, β_j is the effect of perennial ryegrass cultivar, $(\alpha\beta)_{ij}$ is the interaction between the presence of white clover and perennial ryegrass cultivar, and ε_{ijk} is the error.

Differences in chemical composition and digestibility among morphological components (sub-sub-factor) and the interactions with the presence of white clover and perennial ryegrass cultivar were analysed using a split-split-plot model:

$$y = \mu + \gamma_k + \alpha_i + \delta_{ik} + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijk} + \epsilon_l + (\alpha\epsilon)_{il} + (\beta\epsilon)_{jl} + (\alpha\beta\epsilon)_{ijl} + \varepsilon_{ijkl} ,$$

where ϵ_l is the effect of morphological component, their interactions are in the parenthesis marks and $\varepsilon_{i\cdot}$ is the error.

The main effects of white clover and perennial ryegrass cultivar on herbage nutritive value were analysed in the first model, and they were included in the second model for their interactions with morphological component only.

3.3 Results

3.3.1 Morphology

Growing perennial ryegrass with white clover did not significantly change the morphological characteristics of perennial ryegrass cultivars (*P* > 0.05). The tetraploid cultivars, Base and Bealey, had more lamina than diploid cultivars (*P* < 0.05) at vegetative stages (51.7% vs. 45.1% at the pre-heading vegetative stage; 55.0% vs. 50.7% at post-flowering vegetative stage, Table 3.2). At the reproductive stage, the reproductive stem accounted for around 30% of DM. Consequently, the proportion of lamina and pseudostem decreased and the differences among cultivars were not significant (*P* > 0.05).

3.3.2 Nutritive value of morphological components

There were consistent differences in chemical composition and digestibility among morphological components, despite significant interactions between cultivar and morphological component at the reproductive and post-flowering vegetative stages (Table 3.3, 3.4 and 3.5). Lamina had approximately twice the CP concentration of pseudostem or reproductive stem at all stages (213 vs. 105 g/kg DM at the pre-heading vegetative stage, 222 vs. 104 g/kg DM at the reproductive stage and 326 vs. 168 g/kg DM at the post-flowering vegetative stage). Pseudostem had a greater WSC concentration than lamina (from +112 to +281 g/kg DM) and reproductive stems (from +141 to +177 g/kg DM). Compared with the lamina, the pseudostem had a lower DOMD at the post-flowering vegetative stage (71.1% vs. 72.8%), but a greater DOMD at the pre-heading vegetative stage (82.0% vs. 76.8%) and the reproductive stage (76.0% and 74.7%). The reproductive stem had the lowest DOMD (66.2 %) and the greatest NDF concentration (588 g/kg DM), compared with lamina and pseudostem. Dead material was characterised by the low DOMD (46.5%), the low OM (779 g/kg DM), CP (117 g/kg DM) and WSC (5 g/kg DM) concentrations, and the high ADF (305 g/kg DM) and NDF (582 g/kg DM) concentrations.

3.3.3 Effect of white clover on nutritive value of perennial ryegrass

The proportions of white clover in the total herbage mass did not differ among perennial ryegrass cultivars and accounted for < 10% of DM at all stages (Table 3.6). There was no effect of perennial ryegrass cultivar on the nutritive value of white clover. When white clover was present, perennial ryegrass had a greater CP concentration at the pre-heading (162 vs. 170 g/kg DM, $P < 0.05$) and post-flowering vegetative stages (271 vs. 284 g/kg DM, $P = 0.09$, Table 3.7). The interaction indicated that greater increases were found in perennial ryegrass lamina than pseudostem (Table 3.3 and 3.5). Further, the presence of white clover reduced perennial ryegrass WSC concentration at the pre-heading vegetative stage (280 vs. 268 g/kg DM, $P < 0.05$). At the reproductive stage, significant interactions between perennial ryegrass cultivar and the presence of white clover were detected for the concentrations of WSC, ADF and NDF, and DOMD (Table 3.7 and A.1).

3.3.4 Effect of perennial ryegrass cultivar on nutritive value of perennial ryegrass

Nutritive value varied significantly among perennial ryegrass cultivars. The high-sugar cultivar, AberMagic, had the greatest WSC concentration (304 g/kg DM at the pre-heading vegetative stage, 263 g/kg DM at the vegetative stage and 114 g/kg DM at the post-flowering vegetative stage), followed by One50 and tetraploid cultivars, Base and Bealey. The high-sugar cultivar, AberMagic, thus had the greatest DOMD (81.1% at the pre-heading vegetative stage, 74.5% at the vegetative stage and 74.6% at the post-flowering vegetative stage), followed by tetraploid cultivars, Base and Bealey. Generally, at the pre-heading vegetative and reproductive stage, the intermediate-heading cultivars, Kamo and Commando, had greater ADF and NDF concentrations and lower DOMD than all the other cultivars which are late-heading (Table 3.7).

Table 3.2 Morphological components (%) of perennial ryegrass cultivars growing with and without white clover.

	White clover (WC)			Perennial ryegrass cultivar (PRG)									*P* value		
	Absent	Present	SEM†	Base	Bealey	AberMagic	Alto	Commando	Kamo	One50	Prospect	SEM	WC	PRG	WC × PRG
	Pre-heading vegetative stage														
Lamina	46.2	47.2	1.02	51.0^{a}	52.4^{a}	49.0^{ab}	45.7^{b}	39.2^{c}	41.2^{c}	48.6^{ab}	46.9^{b}	1.34	0.54	< 0.01	0.98
Pseudostem	36.5	35.1	0.45	31.7^{d}	33.0^{cd}	35.5^{bcd}	36.8^{bc}	43.0^{a}	38.7^{b}	33.5^{cd}	34.3^{cd}	1.21	0.12	< 0.01	0.82
Dead material	17.3	17.7	0.58	17.3^{bc}	14.7^{d}	15.5^{cd}	17.5^{bc}	17.8^{abc}	20.2^{a}	17.9^{abc}	18.9^{ab}	1.27	0.68	< 0.01	0.43
	Reproductive stage														
Lamina	39.8	40.4	0.38	39.8	42.0	39.9	39.4	41.0	40.3	36.5	42.1	2.47	0.37	0.82	0.74
Pseudostem	12.5	12.6	0.55	11.3	10.6	12.7	12.2	13.5	13.0	13.5	13.4	1.01	0.94	0.35	0.07
Reproductive stem	30.4	31.2	1.30	34.4	34.0	32.2	30.2	27.4	27.4	32.0	28.7	2.65	0.69	0.38	0.25
Dead material	17.3	15.8	0.73	14.5^{c}	13.4^{c}	15.1^{c}	18.2^{ab}	18.1^{ab}	19.3^{a}	18.0^{ab}	15.8^{bc}	0.87	0.25	< 0.01	0.56
	Post-flowering vegetative stage														
Lamina	50.2	53.5	0.75	55.6^{a}	54.4^{ab}	50.7^{bc}	49.5^{c}	48.9^{c}	54.6^{ab}	49.9^{c}	50.5^{bc}	1.30	0.06	< 0.01	0.87
Pseudostem	23.1	21.9	0.78	21.9	24.2	24.0	23.0	22.0	20.8	22.3	21.8	0.83	0.35	0.07	0.68
Dead material	26.7	24.6	0.99	22.5^{b}	21.4^{b}	25.2^{ab}	27.5^{a}	29.1^{a}	24.6^{ab}	27.8^{a}	27.8^{a}	1.37	0.25	< 0.01	0.67

†SEM, standard error of the mean

Means followed by different superscripts are significantly different by Duncan's multiple range test ($P < 0.05$)

Table 3.3 **Chemical composition (g/kg DM) and digestibility (%) of morphological components of perennial ryegrass cultivars growing with and without white clover at the pre-heading vegetative stage.**

	OM		CP‡		WSC		ADF		NDF		DOMD	
	LA†	PS	LA	PS	LA	PS	LA	PS	LA	PS	LA	PS
White clover (WC)												
Absent	921	950	5.34	4.61	167	421	237	236	469	464	76.9	82.0
Present	918	944	5.37	4.68	162	408	235	233	467	456	76.6	82.1
SEM§	1.2		0.010		3.1		1.6		3.0		0.27	
Perennial ryegrass cultivar (PRG)												
Base	920	945	5.36	4.61	167	418	237	231	463	446	77.0	83.3
Bealey	917	941	5.39	4.67	174	413	224	224	449	437	77.6	83.7
AberMagic	925	952	5.33	4.63	206	440	221	226	454	448	79.1	83.8
Alto	919	942	5.36	4.64	150	397	242	242	475	472	76.1	81.3
Commando	918	952	5.38	4.69	128	409	248	243	494	485	74.5	80.0
Kamo	916	944	5.38	4.70	128	374	250	255	491	506	74.5	77.4
One50	922	952	5.29	4.58	190	444	232	224	459	436	77.9	84.2
Prospect	920	947	5.36	4.65	171	419	230	232	459	453	77.5	82.6
SEM	2.6		0.033		11.3		3.2		5.7		0.63	
Mean	920	947	5.36	4.65	164	414	236	235	468	460	76.8	82.0
SEM	0.7		0.006		2.4		1.1		2.0		0.19	
P value												
Morphological component (MC)	< 0.01		< 0.01		< 0.01		0.56		0.01		< 0.01	
MC × WC	0.25		< 0.01		0.30		0.86		0.27		0.45	
MC × PRG	0.14		0.50		0.06		0.27		0.08		0.06	
MC × WC × PRG	0.71		0.93		0.83		0.93		0.82		0.66	

†LA, lamina; PS, pseudostem
‡Data were ln transformed and back-transformed data are present in Table A.1
§SEM, standard error of the mean

Table 3.4 Chemical composition (g/kg DM) and digestibility (%) of morphological components of perennial ryegrass cultivars growing with and without white clover at the reproductive stage.

	OM			CP‡			WSC			ADF			NDF			DOMD		
	LA†	PS	RS	LA	PS	RS	LA	PS	RS	LA	PS	RS	LA	PS	RS	LA	PS	RS
White clover (WC)																		
Absent	916	936	941	5.35	4.59	4.61	165	329	270	241	269	314	487	509	593	74.4	76.3	65.7
Present	913	931	938	5.43	4.67	4.66	152	307	271	233	268	307	474	512	584	75.0	75.8	66.7
SEM§		3.7			0.046			17.0			3.2			6.3			0.70	
Perennial ryegrass cultivar(PRG)																		
Base	919	938	945	5.31	4.52	4.54	185	362	316	237	256	300	477	486	571	75.7	78.8	68.5
Bealey	909	930	940	5.44	4.63	4.63	148	316	302	231	261	288	474	496	553	74.3	76.7	69.7
AberMagic	921	940	948	5.36	4.59	4.61	187	353	322	225	263	297	459	503	571	77.8	77.5	69.5
Alto	918	932	938	5.34	4.57	4.63	168	323	267	239	272	313	476	512	596	75.2	76.1	66.0
Commando	907	924	933	5.51	4.81	4.71	122	263	205	240	276	338	491	532	631	72.7	74.1	61.1
Kamo	912	927	931	5.43	4.65	4.75	148	292	180	243	279	341	491	524	633	73.7	74.4	60.6
One50	921	942	942	5.33	4.54	4.59	178	352	292	237	263	301	479	497	572	75.2	77.0	67.0
Prospect	912	933	940	5.41	4.70	4.61	132	282	281	247	278	304	497	532	577	72.9	73.7	67.2
SEM		3.1			0.042			15.7			5.0			8.7			0.91	
Mean	915[c]	933[b]	940[a]	5.39[a]	4.63[b]	4.63[b]	158[b]	318[a]	271[b]	237[c]	269[b]	310[a]	480[c]	510[b]	588[a]	74.7[b]	76.0[a]	66.2[c]
SEM		0.8			0.009			4.3			1.6			2.8			0.28	
P value																		
Morph. Comp. (MC)		< 0.01			< 0.01			< 0.01			< 0.01			< 0.01			< 0.01	
MC × WC		0.75			0.25			0.19			0.21			0.11			0.13	
MC × PRG		0.28			0.02			< 0.01			< 0.01			< 0.01			< 0.01	
MC × WC × PRG		0.61			0.85			0.69			0.66			0.53			0.50	

†LA, lamina; PS, pseudostem; RS, reproductive stem

‡Data were ln transformed and back-transformed data are present in Table A.1

§SEM, standard error of the mean

Means followed by different superscripts are significantly different by Duncan's multiple range test ($P < 0.05$)

Table 3.5 Chemical composition (g/kg DM) and digestibility (%) of morphological components of perennial ryegrass cultivars growing with and without white clover at the post-flowering vegetative stage.

	OM		CP		WSC		ADF		NDF		DOMD	
	LA†	PS	LA	PS	LA	PS	LA	PS	LA	PS	LA	PS
White clover (WC)												
Absent	897	891	320	166	37	171	258	305	482	540	72.7	71.6
Present	898	889	331	170	39	181	247	293	464	524	73.7	72.9
SEM§	1.0		3.7		3.0		3.8		5.7		0.49	
Perennial ryegrass cultivar (PRG)												
Base	896	885	335	169	38	163	253	301	473	530	73.6	72.1
Bealey	895	885	339	169	45	192	244	287	458	510	74.1	73.9
AberMagic	902	896	308	155	55	237	252	283	471	507	74.6	74.7
Alto	896	890	316	158	38	172	259	312	479	548	72.1	71.2
Commando	899	889	322	169	28	149	255	312	479	555	72.4	70.3
Kamo	900	890	324	170	38	169	251	297	472	533	73.4	72.1
One50	898	896	325	168	35	184	257	300	482	534	72.7	72.6
Prospect	895	887	335	185	29	141	250	299	470	539	72.8	71.1
SEM	1.7		4.5		8.4		3.8		6.5		0.53	
Mean	897	890	326	168	38	176	253	299	473	532	73.2	72.3
SEM	0.7		0.9		2.5		1.2		2.1		0.15	
P value												
Morphological component (MC)	< 0.01		< 0.01		< 0.01		< 0.01		< 0.01		< 0.01	
MC × WC	0.14		0.04		0.25		0.62		0.80		0.46	
MC × PRG	0.35		0.01		< 0.01		0.03		0.05		0.08	
MC × WC × PRG	0.96		0.70		0.85		0.80		0.47		0.32	

†LA, lamina; PS, pseudostem

§SEM, standard error of the mean

Table 3.6 Proportion (%), chemical composition (g/kg DM) and digestibility (%) of white clover growing with perennial ryegrass cultivars.

	Base	Bealey	AberMagic	Alto	Commando	Kamo	One50	Prospect	SEM†	*P* value
	Perennial ryegrass pre-heading vegetative stage									
Proportion	5.7	8.2	7.4	4.6	6.1	7.2	4.7	7.6	1.86	0.79
OM	911	912	908	905	906	911	909	911	3.4	0.77
CP	313	319	323	317	309	318	293	308	6.6	0.11
WSC	130	127	114	112	109	117	131	126	6.5	0.14
ADF	187	178	185	189	191	193	187	191	4.6	0.44
NDF	266	273	272	282	290	271	269	285	7.7	0.33
DOMD	79.2	79.3	79.5	78.3	78.2	79.4	79.0	78.8	0.58	0.67
	Perennial ryegrass reproductive stage									
Proportion	4.2	10.7	10.1	4.8	4.2	9.5	3.7	7.1	1.99	0.08
OM	900	898	899	893	898	893	900	903	3.4	0.44
CP	271	303	287	299	275	287	279	274	9.7	0.21
WSC	138	109	137	111	125	121	113	139	11.3	0.32
ADF	197	192	190	195	205	200	195	207	4.4	0.14
NDF	284	298	295	310	317	306	314	299	9.4	0.27
DOMD	77.4	76.9	77.3	75.9	75.5	76.0	76.6	76.9	0.75	0.55
	Perennial ryegrass post-flowering vegetative stage									
Proportion	5.2	6.7	6.3	3.3	3.4	5.1	3.5	5.6	1.65	0.70
OM	888	888	888	892	886	893	893	892	2.9	0.53
CP	349	357	354	365	362	357	356	345	5.8	0.33
WSC	64	66	70	69	57	67	75	66	5.3	0.52
ADF	198	193	195	190	191	192	191	203	3.8	0.25
NDF	253	242	249	240	267	251	240	262	7.3	0.13
DOMD	76.7	77.0	76.9	78.0	76.6	78.0	78.2	77.1	0.52	0.19

†SEM, standard error of the mean

Table 3.7 Chemical composition (g/kg DM) and digestibility (%) of perennial ryegrass cultivars growing with and without white clover

	White clover (WC)			Perennial ryegrass cultivar (PRG)									*P* value		
	Absent	Present	SEM†	Base	Bealey	AberMagic	Alto	Commando	Kamo	One50	Prospect	SEM	WC	PRG	WC × PRG
	Pre-heading vegetative stage														
OM	934	930	0.9	930^{abc}	926^{c}	937^{a}	929^{bc}	936^{ab}	930^{bc}	935^{ab}	932^{abc}	2.2	0.05	0.02	0.25
CP	162	170	1.5	172	176	165	164	161	165	158	167	5.3	0.03	0.39	0.29
WSC	280	268	2.6	264^{bc}	267^{bc}	304^{a}	261^{bc}	276^{abc}	247^{c}	295^{ab}	276^{abc}	10.6	0.05	0.01	0.24
ADF	236	234	1.1	235^{c}	224^{de}	223^{e}	242^{b}	245^{b}	252^{a}	229^{cde}	231^{cd}	2.3	0.21	< 0.01	0.11
NDF	467	463	2.2	456^{c}	444^{d}	451^{cd}	475^{b}	489^{a}	498^{a}	449^{cd}	457^{c}	4.0	0.26	< 0.01	0.32
DOMD	79.2	78.9	0.16	79.4^{bc}	80.0^{ab}	81.1^{a}	78.4^{cd}	77.4^{d}	75.9^{e}	80.5^{ab}	79.7^{abc}	0.47	0.42	< 0.01	0.26
	Reproductive stage														
OM	928	925	3.5	932^{ab}	924^{cd}	934^{a}	927^{abc}	919^{d}	921^{cd}	932^{ab}	925^{bcd}	2.5	0.55	< 0.01	0.19
CP	156	167	7.7	145^{d}	167^{abc}	155^{bcd}	156^{bcd}	184^{a}	171^{ab}	147^{cd}	167^{abc}	6.7	0.39	< 0.01	0.29
WSC	227	220	6.0	260^{a}	231^{ab}	263^{a}	225^{ab}	172^{d}	181^{cd}	249^{a}	209^{bc}	10.7	0.76	< 0.01	0.04
ADF	273	266	2.2	266^{bcd}	257^{d}	258^{cd}	272^{abc}	279^{ab}	283^{a}	268^{bcd}	271^{abcd}	4.6	0.12	< 0.01	0.06
NDF	530	520	4.5	518^{b}	507^{b}	509^{b}	527^{ab}	545^{a}	545^{a}	520^{b}	530^{ab}	7.3	0.23	< 0.01	0.05
DOMD	71.4	72.0	0.49	73.1^{ab}	72.8^{ab}	74.5^{a}	71.8^{b}	69.1^{c}	69.3^{c}	72.1^{b}	71.1^{bc}	0.76	0.45	< 0.01	0.02
	Post-flowering vegetative stage														
OM	895	895	0.7	893^{c}	892^{c}	900^{a}	894^{bc}	895^{bc}	897^{ab}	898^{ab}	892^{c}	1.3	0.06	< 0.01	0.14
CP	271	284	3.6	288^{ab}	286^{ab}	259^{d}	266^{cd}	274^{bc}	282^{ab}	274^{bc}	290^{a}	4.6	0.09	< 0.01	0.61
WSC	80	81	1.5	73^{bc}	90^{b}	114^{a}	81^{bc}	64^{c}	74^{bc}	82^{bc}	63^{c}	5.8	0.60	< 0.01	0.64
ADF	273	261	3.2	266^{bc}	257^{d}	262^{cd}	276^{a}	274^{a}	264^{bcd}	271^{ab}	265^{bcd}	2.5	0.08	< 0.01	0.27
NDF	500	481	2.2	489^{bc}	474^{d}	483^{cd}	501^{ab}	503^{a}	489^{bc}	498^{ab}	490^{abc}	10	0.07	< 0.01	0.55
DOMD	72.4	73.5	0.41	73.1^{bc}	74.0^{ab}	74.6^{a}	71.8^{d}	71.6^{d}	73.0^{bc}	72.6^{cd}	72.3^{cd}	0.38	0.15	< 0.01	0.28

†SEM, standard error of the mean

Means followed by different superscripts are significantly different by Duncan's multiple range test ($P < 0.05$)

3.4 Discussion

3.4.1 Morphology of perennial ryegrass

This study provides information on key morphological characteristics of perennial ryegrass affected by the presence of white clover and cultivar. The presence of white clover exerted little effect on the morphological characteristics of perennial ryegrass, such as the proportion of the lamina, pseudostem, reproductive stem and dead material. This may reflect a low overall proportion of white clover (< 10% DM above ground level) and thus its little opportunity to affect perennial ryegrass morphology. Further, it is noted that quantitative trait loci are responsible for morphological traits in perennial ryegrass, including plant height, tiller size, leaf length and leaf width, with little environmental impact (Yamada et al. 2004). Therefore, perennial ryegrass's morphology remains stable no matter growing with or without white clover.

As for the cultivar effect, a key result was that lamina proportions were greater in tetraploid than diploid cultivars at the vegetative stage, which agrees with the previous study (Orr et al. 2004). At the reproductive stage, the emergence of inflorescences reduced the proportions of both lamina and pseudostem and thus diminished their differences among cultivars. Similarly, Laidlaw (2004) found that cultivars of different heading dates expressed no significant differences in leaf lamina proportion after heading. In this study, under rotational grazing, intermediate-heading perennial ryegrass cultivars, Kamo and Commando, did not produce more reproductive stems than late-heading cultivars, but their reproductive stems had greater ADF and NDF concentrations, suggesting that they were more mature.

3.4.2 Nutritive value of perennial ryegrass morphological components

There were interactions between morphological component and cultivar for nutritive value, indicating that the nutritive value differences among cultivars were different in each morphological component. To be specific, greater differences in the CP concentration among cultivars were found in lamina than pseudostem and reproductive stem, while the variation in the WSC concentration was greater in pseudostem than lamina. Despite the interactions, lamina consistently had a greater CP and a lower WSC concentration than pseudostem, which agreed with previous studies (Waite & Boyd 1953; Chaves et al. 2006b). These findings showed that a greater variation in the concentration of a certain nutrient among cultivars would be found in the plant part that had a greater concentration of this nutrient.

In terms of digestibility, pseudostem had similar or greater DOMD than lamina. This agrees with previous results (Poppi et al. 1981) and could be explained by the greater veins distance in the sheaths than the leaf blades (Wilman et al. 1996). WSC are highly digestible (Heeren et al. 2014) and accumulates in the leaf sheaths (Fulkerson & Donaghy 2001), which could also contribute to a greater digestibility of the pseudostem. In practice, the dead material attached to the sheaths, making the digestibility low in the lower sward layer. In terms of feeding value, which is a combination of herbage nutritive value and voluntary intake (Chapman et al. 2014), lamina is still greater than pseudostem, because pseudostems are hard to access (Chapman et al. 2012) and require more breaking force to be ingested (Bryant et al. 2008). Therefore, it is still necessary to increase lamina proportion and depress the amount of dead material through pasture management to achieve a higher herbage feeding value (Casey et al. 1999).

3.4.3 Effect of white clover on nutritive value of perennial ryegrass

Including white clover into perennial ryegrass pastures led to a greater CP concentration and lower ADF and NDF concentrations of overall herbage (Chen et al. 2016). This was due to a high CP concentration and a low fibre concentration of white clover than perennial ryegrass (Evans et al. 1996). Additionally, in this study, the presence of white clover increased the CP concentration in the accompanying perennial ryegrass at two vegetative stages. This could be a consequence of the N transfer from legumes to grass via decay of plant tissues, root exudation of ammonium and amino acid and mycorrhizal hyphae (Paynel et al. 2008). Interactions between white clover and morphological components indicated that lamina responded more sensitively to the presence of white clover than pseudostem.

At the reproductive stage, the presence of white clover resulted in lower herbage WSC concentrations in Base, Alto, Kamo and Prospect, but greater herbage WSC concentrations in Bealey, AberMagic, Commando and One50. Fibre concentrations (ADF and NDF) decreased in Bealey, AberMagic, Commando, Kamo and One50, with greater digestibility when white clover was included. In contrast, other cultivars were the opposite. These interactions indicated that growing with white clover had different effects on nutritive value for different perennial ryegrass cultivars at the reproductive stage. The reason for these interactions is not clear, but it may be related to the interactions between perennial ryegrass cultivars' reproductive growth and external N input (Bahmani et al. 2001b). However, the interaction only occurred at the reproductive stage and the magnitude was small.

3.4.4 Effect of cultivar on nutritive value of perennial ryegrass

In this study, the high-sugar cultivar, AberMagic, had a higher nutritive value, as determined by the greatest herbage WSC concentration and the greatest DOMD with low ADF and NDF concentrations. Tetraploid cultivars, Base and Bealey, followed, while the intermediate-heading cultivars, Commando and Kamo, had a relatively low herbage nutritive value. These findings confirmed that high-sugar cultivars (Miller et al. 2001; Lee et al. 2002; Moorby et al. 2006), tetraploid cultivars (Wims et al. 2013; Solomon et al. 2014), late-heading cultivars (Gowen et al. 2003) had a higher nutritive value and the potential to increase milk production, compared with their counterparts.

Herbage nutritive value varied in different seasons and at different regrowth stages, and this was partly due to the changes in the ratio of green leaf, stem and dead material, which are different in chemical composition and digestibility (Stewart & Hayes 2011). However, the results suggested that the differences in morphology (such as lamina proportion) is not the main reason why perennial ryegrass cultivars differed in nutritive value. There are three findings supporting this point. Firstly, nutritive value varied among cultivars within the same morphological component (Table 3, 4 and 5). For example, the high-sugar cultivar (i.e. AberMagic) had a greater WSC concentration than all the other cultivars in all morphological components, including the lamina, pseudostem and reproductive stem. Secondly, it was found that lamina had a greater CP concentration and a lower WSC concentration than pseudostem. However, cultivars with greater proportions of lamina did not necessarily have greater overall herbage CP concentrations, while a greater proportion of pseudostem did not lead to a greater overall herbage WSC concentration. For example, tetraploid cultivars had greater lamina proportions, but their CP concentrations were not always greater than diploid cultivars. For another, Commando and Kamo had the greatest proportions of pseudostem at the pre-heading vegetative stage, but their WSC concentrations were less than AberMagic. Finally, when morphological proportions were similar among cultivars at the reproductive stage, the chemical composition of cultivars was still different regardless. Therefore nutritive value variation among cultivars, in part, reflected the genetic variation in metabolism of nutrient synthesis and turnover (Wilkins et al. 2000), but not the morphology.

3.5 Conclusion

The presence of white clover had little effect on morphological characteristics but could result in a greater CP concentration of accompanying perennial ryegrass, especially in the lamina fraction at the vegetative stage. Lamina proportions were greater in tetraploid cultivars and diploid cultivars. At the reproductive stage, the emergence of reproductive stems with a lower digestibility diminished the differences in morphology among cultivars and led to a lower digestibility. Lamina consistently had a greater CP and a lower WSC concentration than pseudostem. The high-sugar diploid cultivar, AberMagic, and tetraploid cultivars, Base and Bealey, had a greater WSC concentrations, lower ADF and NDF concentrations and a greater digestibility than the other cultivars. However, morphology is not the main reason why perennial ryegrass cultivars differ in nutritive value.

Chapter 4

Nutritive value variation of perennial ryegrass cultivars growing with and without white clover harvested at different times of day during pasture regrowth

4.1 Introduction

Perennial ryegrass (*Lolium perenne* L.) is widely used for dairy production in temperate regions (Humphreys et al. 2010). A range of cultivars have been developed for better herbage dry matter (DM) yield, heading date, nutritive value and persistence (Stewart & Hayes 2011; Lee et al. 2012). However, pasture management factors, such as growing perennial ryegrass in a monoculture or a mixture, the defoliation interval and pasture allocation timing, could have more pronounced and consistent effects on herbage nutritive value than perennial ryegrass cultivars (Francis et al. 2006; Cosgrove et al. 2007; Burke et al. 2011; Bryant et al. 2012).

Growing perennial ryegrass with white clover (*Trifolium repens* L.) or other legumes is a common practice in temperate pastures (Peoples & Baldock 2001) as an alternative to nitrogen (N) fertiliser application (Andrews et al. 2007). In mixtures, perennial ryegrass is one of the biological components forming the pasture ecosystem. The effect of other components and the interactions between components could either outweigh or diminish the differences among perennial ryegrass cultivars (Hyslop et al. 2000; Lee et al. 2012). However, these interactions have not usually been considered in the perennial ryegrass breeding or evaluations (Stewart & Hayes 2011).

When pastures are managed under rotational grazing, herbage accumulates at the expense of digestibility during pasture regrowth, with an increasing herbage fibre concentration (Mambrini et al. 1994; Steg et al. 1994; Ayres et al. 1998). During the day, water-soluble carbohydrates (WSC) accumulate (Orr et al. 2001b) and were generally accompanied by lower herbage neutral detergent fibre (NDF) and acid detergent fibre (ADF) concentrations (Gregorini et al. 2009; Sauvé et al. 2009). Timing of defoliation (at different regrowth stages and times of day) is thus critical to balance herbage DM yield and nutritive value. The variation in nutritive value at different regrowth stages and times of day has been studied in a range of forage plant species (Ayres et al. 1998; Chaves et al. 2006a; Shewmaker et al. 2006). However, few studies have investigated nutritive value variation during pasture regrowth and time of day simultaneously or focused on the interactions between the timing of defoliation and perennial ryegrass cultivar. Previously, Turner et al. (2015) found that the differences in herbage WSC concentration between high-sugar and normal-sugar cultivars were significantly greater at a later regrowth stage, compared with the earlier stages.

The objectives of this study were (1) to define the effects of perennial ryegrass cultivar and the presence of white clover on pasture herbage nutritive value at different times of day during pasture regrowth and (2) to clarify the interactions among perennial ryegrass cultivar, the presence of white clover, regrowth stage and time of day.

4.2 Materials and Methods

Three perennial ryegrass cultivars (AberMagic AR1, a diploid high-sugar cultivar; Bealey NEA2, a tetraploid cultivar; Prospect AR37, a normal-sugar diploid cultivar) were evaluated for nutritive value variation. These three cultivars were selected out of the eight cultivars in Chapter 3, representing a tetraploid (Bealey) vs. diploid cultivars (AberMagic and Prospect) comparison, and a high-sugar (AberMagic) vs. normal-sugar cultivars (Prospect) comparison. This study was conducted within the experiment described in 3.2.1 and 3.2.2. It was a sequential split-plot design with four blocks under a high N fertiliser application rate (325 kg N/ha). The main-plot factor was the presence of white clover, the sub-plot factor was perennial ryegrass cultivar, the sub-sub-plot factor was regrowth stage and the sub-sub-sub-plot factor was time of day.

4.2.1 Sampling and analysis

In order to have a similar initial residual for regrowth, all plots were mown to 4 cm above ground level after the previous grazing on 18 March 2015. Herbage samples were taken 7, 14, 21 and 28 days after defoliation. On each sampling day, four samples were collected during the day from sunrise (around 0715 h) to sunset (around 1745 h). Herbage samples of 100 g fresh weight were cut at grazing level (4 cm above ground level) from at least six random spots in each sub-plot. Fresh herbage samples were placed in a chilly bin immediately, transferred to the laboratory as soon as possible and separated into perennial ryegrass and white clover (if applicable). Perennial ryegrass and white clover herbage samples were oven-dried at 60°C for 48 h and the proportion of white clover were calculated on a DM basis. Oven-dried perennial ryegrass and white clover herbage were ground through a 1-mm sieve separately. Ground samples were analysed for the concentrations of CP, NDF and WSC and organic matter digestibility in dry matter (DOMD) by near infrared reflectance spectroscopy (NIRS Systems 5000, Foss, Maryland, USA). NIRS calibrations were previously derived on perennial ryegrass samples (Jones & Hayward 1975; MAFF 1986; AOAC 1990; van Soest et al. 1991). Assays for NDF excluded sodium sulphite or α-amylase and NDF predictions were inclusive of residual ash (Bryant et al. 2012). The chemical composition and digestibility of the mixtures were calculated according to the proportion of white clover and perennial ryegrass.

4.2.2 Statistical analysis

ANOVA was performed with SPSS (version 22). The effect of perennial ryegrass cultivar on the proportion of white clover in each regrowth stage was tested by two-way ANOVA, using the model: $y = \mu + \gamma_k + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijk}$,

where y is the dependent variable, μ is the overall mean, α_i is the effect of regrowth stage, γ_k is the block effect, β_j is the effect of perennial ryegrass cultivar, $(\alpha\beta)_{ij}$ is the interaction between regrowth stage and perennial ryegrass cultivar and ε_{ijk} is the residual error.

The effects of white clover, perennial ryegrass cultivar, regrowth stage, time of day and their interactions on herbage chemical composition and digestibility were analysed by a sequential split-plot model:

$$y = \mu + \alpha_i + \gamma_k + \varepsilon_{ik} + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijk} + \epsilon_l + (\alpha\epsilon)_{il} + (\beta\epsilon)_{jl} + (\alpha\beta\epsilon)_{ijl} + \varepsilon_{ijkl} + \theta_n + (\alpha\theta)_{in} + (\beta\theta)_{jn} + (\epsilon\theta)_{ln} + (\alpha\beta\theta)_{ijn} + (\alpha\epsilon\theta)_{iln} + (\beta\epsilon\theta)_{jln} + (\alpha\beta\epsilon\theta)_{ijln} + \varepsilon_{ijkln},$$

where y is the dependent variable, μ is the overall mean, α_i is the effect of white clover, γ_k is the block effect, β_j is the effect of perennial ryegrass cultivar, ϵ_l is the effect of regrowth stage, θ_n is the effect of time of day, the interactions are in the parenthesis marks and $\varepsilon_{i.}$ is the residual error.

To further analyse the nutritive value variation, quadratic polynomial regressions ($z = z_0 + ax + by + cx^2 + dy^2 + fxy$) were performed on herbage nutritive value (z, the concentration of WSC, CP and NDF and DOMD) changing at different regrowth stages (x, from 7 to 28 d) and times of day (y, from 0 to 1) using OriginLab (version 8.6). y = 0 represented the sunrise and y = 1 represented the sunset. Parameters are shown in Table A.2.

4.3 Results

4.3.1 Proportion of white clover

White clover proportion above grazing level increased significantly ($P < 0.01$) from 7.9% on day 7 to 12.4% on day 28 of regrowth. At all regrowth stages, white clover proportion was always the greatest in AberMagic ($P < 0.05$, Table 4.1), peaking at 13.8% on day 28 of regrowth. White clover proportions were similar in Bealey and Prospect (11.9% vs. 11.4% on day 28, $P > 0.05$). There was no interaction ($P > 0.05$) between perennial ryegrass cultivar and regrowth stage for the proportion of white clover.

4.3.2 Herbage nutritive value

The high-sugar cultivar, AberMagic, had the greatest WSC concentration ($P < 0.01$, Table 4.2), followed by Bealey and Prospect (averaged at 162 g/kg DM, 144 g/kg DM and 113 g/kg DM, respectively), in both perennial ryegrass monocultures and mixtures with white clover (Figure 4.1, Table A.3). The differences in herbage WSC concentration among cultivars increased during pasture regrowth (Figure 4.1, $P < 0.01$ for the interaction). Herbage WSC concentration increased during pasture regrowth from 87 g/kg DM on day 7 to 186 g/kg DM on day 28, and during the day from 93 g/kg DM at sunrise to 180 g/kg DM at sunset. These increases were greater in perennial ryegrass monocultures than perennial ryegrass-white clover mixtures (Figure 4.1, $P < 0.01$ for both interactions).

No significant differences were detected among perennial ryegrass cultivars in the CP concentration ($P > 0.05$, Table 4.2). However, compared with perennial ryegrass monocultures, perennial ryegrass-white clover mixtures had a greater CP concentration ($P < 0.05$, Table 4.2). Further, the differences were greater at later regrowth stages (+44 g/kg DM on day 28) than earlier stages (+11 g/kg DM on day 7, Table A.4). The presence of white clover led to a lower NDF concentration (421 vs. 397 g/kg DM) in all cultivars. Herbage CP concentration decreased during pasture regrowth ($P < 0.01$) from 290 g/kg DM on day 7 to 239 g/kg DM on day 28 with a greater rate in Bealey from 301 to 241 g/kg DM ($P < 0.01$ for the interaction, Figure 4.2). Herbage CP concentration was greater ($P < 0.01$) in the morning than the afternoon and the difference increased as the regrowth proceeded (Figure 4.2, $P < 0.01$ for the interaction).

Herbage NDF concentration was affected by both the presence of white clover ($P < 0.01$) and perennial ryegrass cultivar ($P < 0.01$, Table 4.2). Prospect always had the greatest NDF concentration (426 vs. 404 and 403 g/kg DM for AberMagic and Bealey, respectively). Compared with Bealey, AberMagic had a greater NDF concentration at the early regrowth stages (day 7 and 14) but a lower NDF concentration at the late regrowth stages (day 21 and 28) (Figure 4.3, $P < 0.01$ for the interaction). The NDF concentration increased during pasture regrowth ($P < 0.01$) from 394 g/kg DM on day 7 to 436 g/kg DM on day 28, and decreased during the day (*P* < 0.01) from 439 g/kg DM at sunrise to 378 g/kg DM at sunset. A significant interaction (*P* < 0.01) between regrowth stage and time of day indicated larger diurnal changes in NDF concentration at later regrowth stages (Figure 4.3).

Compared with the NDF concentration, DOMD showed a reverse pattern (Figure 4.4, Table A.6). DOMD of Prospect (77.6%) was always lower (*P* < 0.01) than AberMagic (80.0%) and Bealey (79.5%). DOMD decreased during pasture regrowth (*P* < 0.01) from 81.4% on day 7 to 75.1% on day 28, and increased during the day (*P* < 0.01) from 76.4% at sunrise to 81.2% at sunset. The presence of white clover did not significantly affect the herbage DOMD (P > 0.05), but it reduced the differences in DOMD among perennial ryegrass cultivars at different regrowth stages (P < 0.05 for the interaction) and times of day (P < 0.05 for the interaction). Similar to the NDF concentration, greater diurnal variation in DOMD (*P* < 0.01) was detected at later regrowth stages (Figure 4.4).

Table 4.1 White clover proportion (%) in perennial ryegrass pastures during pasture regrowth.

Perennial ryegrass cultivar	Regrowth stage (day)				Mean	Regrowth		Cultivar		Regrowth × cultivar	
	7	14	21	28		SEM†	*P* value	SEM	*P* value	SEM	*P* value
AberMagic	9.5	11.9	12.8	13.8	12.0[a]	0.76	0.02	0.66	0.05	1.32	0.99
Bealey	6.5	10.0	11.0	11.9	9.8[b]						
Prospect	7.8	10.0	10.7	11.4	10.0[b]						
Mean	7.9[b]	10.6[a]	11.5[a]	12.4[a]							

†SEM, standard error of the mean

Means followed by different superscripts are significantly different by Duncan's multiple range test ($P < 0.05$)

Table 4.2 Effects of white clover, perennial ryegrass cultivar, regrowth stage and time of day on chemical composition and digestibility of perennial ryegrass pastures.

Effect	degree of freedom	CP	WSC	NDF	DOMD
White clover (WC)	1	0.03	0.14	< 0.01	0.40
Perennial ryegrass cultivar (PRG)	2	0.21	< 0.01	< 0.01	< 0.01
WC × PRG	2	0.96	0.98	0.71	1.00
Regrowth (R)	3	< 0.01	< 0.01	< 0.01	< 0.01
Time of day (T)	3	< 0.01	< 0.01	< 0.01	< 0.01
WC × R	3	< 0.01	< 0.01	< 0.01	< 0.01
PRG × R	6	< 0.01	< 0.01	< 0.01	0.01
WC × T	3	0.11	0.04	1.00	0.01
PRG × T	6	0.84	0.81	0.89	0.90
R × T	9	< 0.01	< 0.01	< 0.01	< 0.01
WC × PRG × R	6	0.26	0.08	0.15	0.05
WC × PRG × T	6	0.92	0.70	0.91	0.88
WC × R× T	9	0.81	0.83	0.53	0.21
PRG × R × T	18	0.43	0.89	0.38	0.31
WC × PRG × R × T	18	0.93	0.78	0.87	0.84

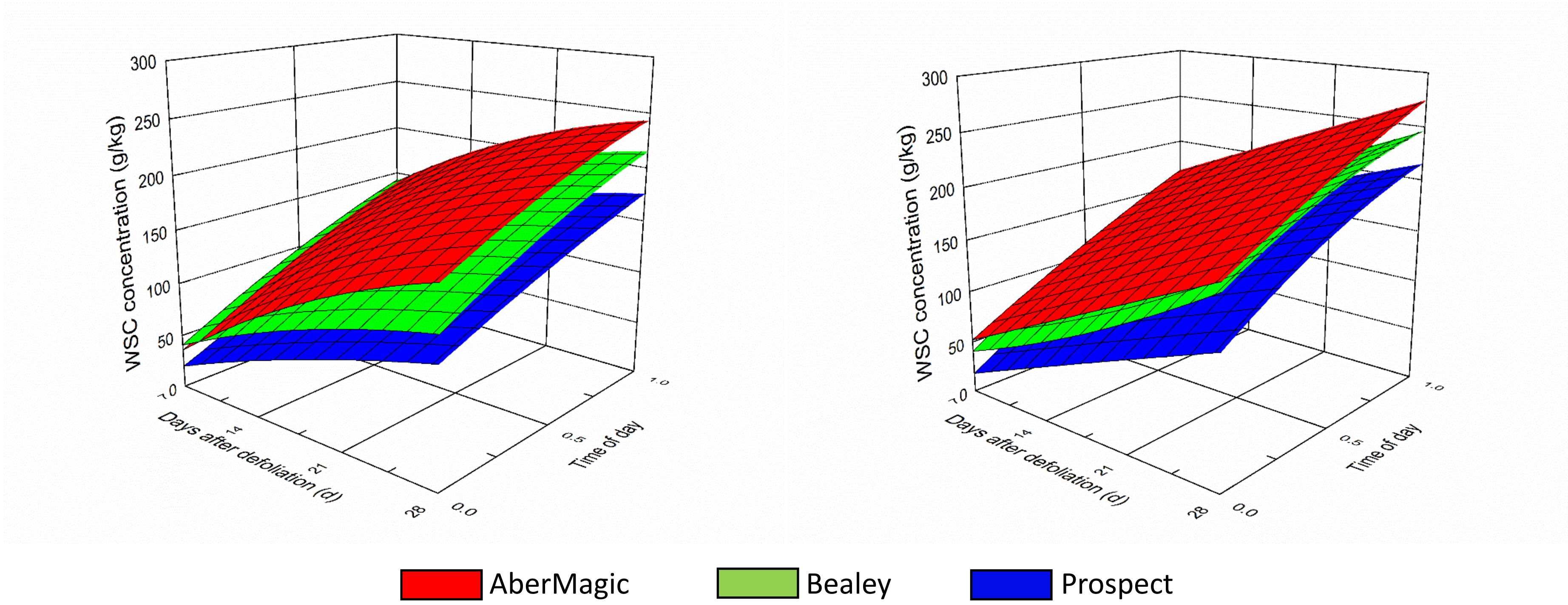


Figure 4.1 Variation in the WSC concentration of perennial ryegrass cultivars growing with (left) and without (right) white clover at different regrowth stages and times of day (0.0: sunrise; 1.0: sunset).

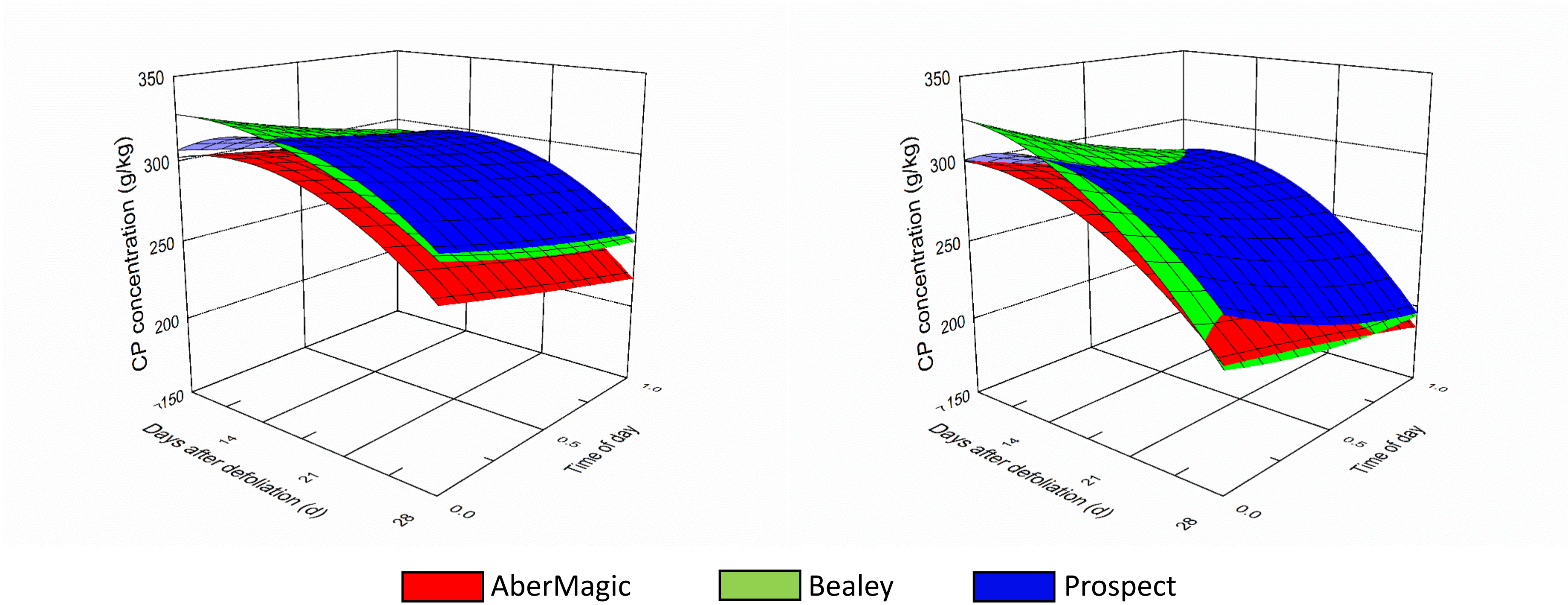


Figure 4.2 Variation in the CP concentration of perennial ryegrass cultivars growing with (left) and without (right) white clover at different regrowth stages and times of day (0.0: sunrise; 1.0: sunset).

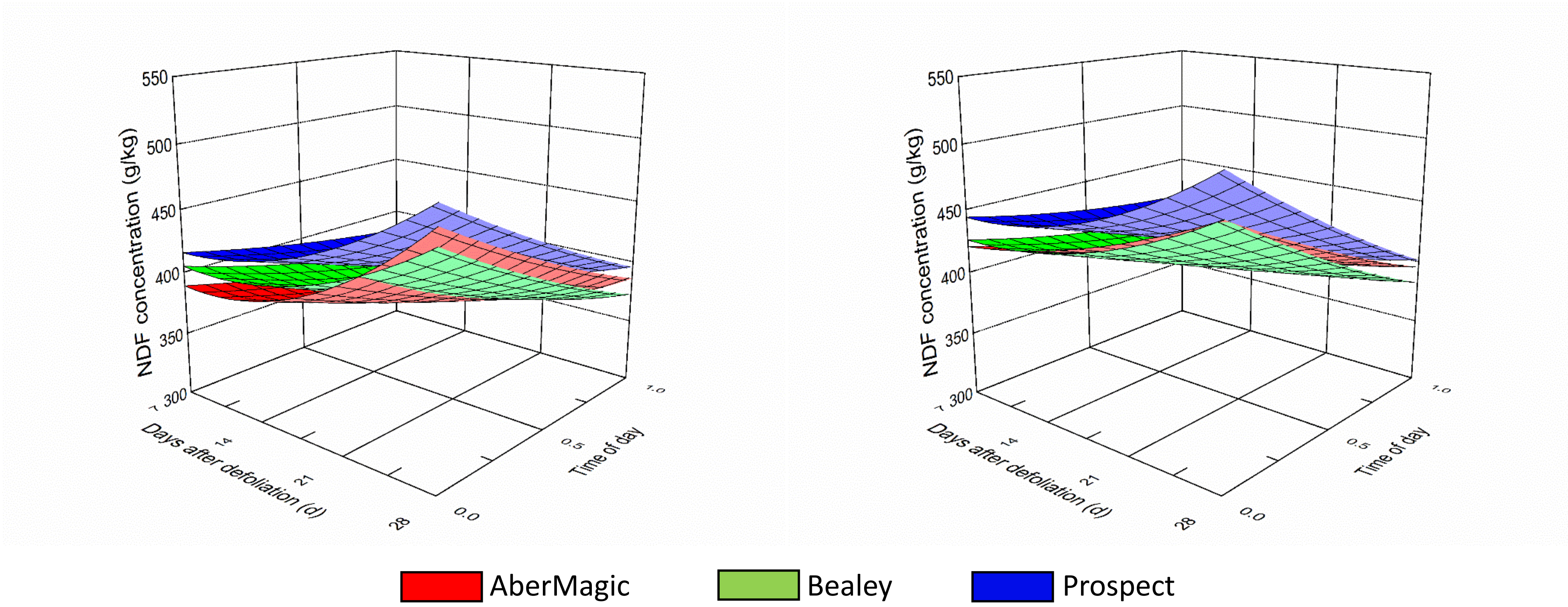


Figure 4.3 Variation in the NDF concentration of perennial ryegrass cultivars growing with (left) and without (right) white clover at different regrowth stages and times of day (0.0: sunrise; 1.0: sunset).

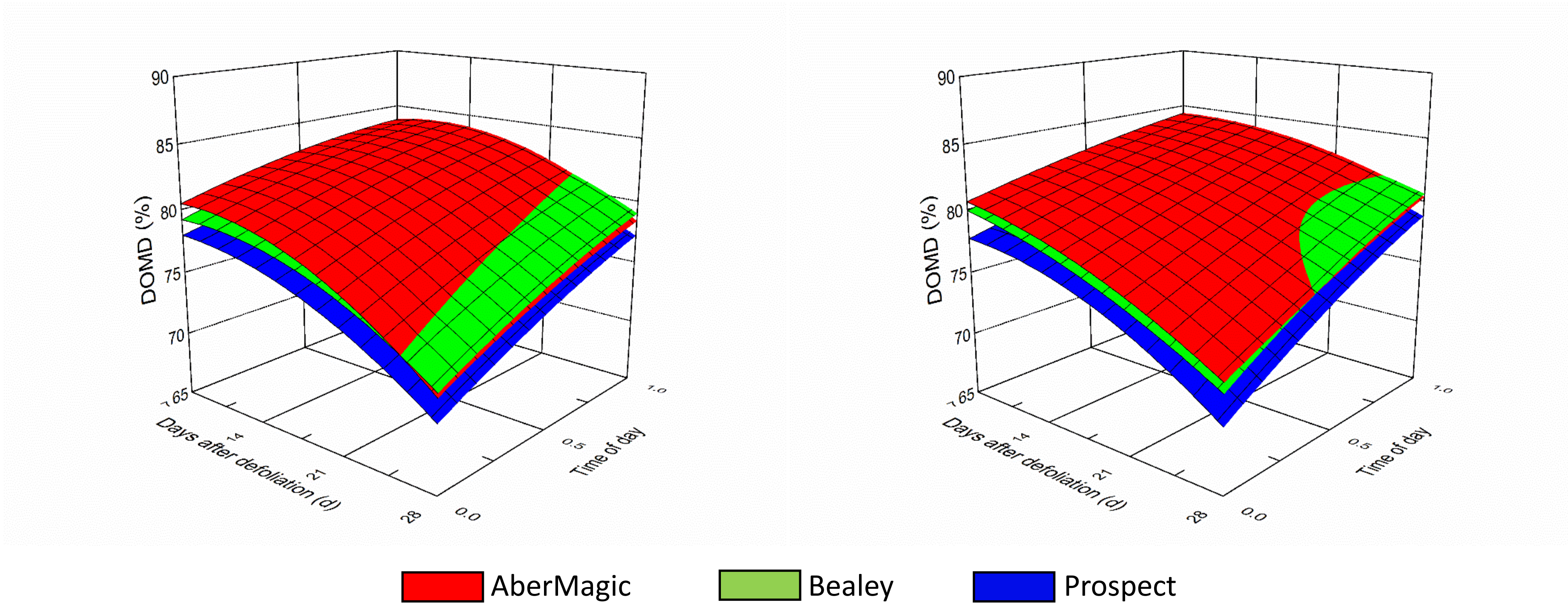


Figure 4.4 Variation in DOMD of perennial ryegrass cultivars growing with (left) and without (right) white clover at different regrowth stages and times of day (0.0: sunrise; 1.0: sunset).

4.4 Discussion

4.4.1 The presence of white clover

In this study, the proportion of white clover increased above grazing level during pasture regrowth with a greater increase found from day 7 to day 14. This is consistent with a previous study (Ryle et al. 1985), which reported that new white clover leaves grew following an exponential pattern after defoliation. A greater white clover proportion was recorded in the high-sugar cultivar, AberMagic. The explanation for this is not clear. One possible reason could be the fact that dairy cows prefer cultivars with elevated WSC concentrations (Smit et al. 2006; Chen et al. 2016), thus leading to more intensive grazing, which would encourage more white clover to grow in their open habitat.

In terms of nutritive value, the presence of white clover in mixtures significantly improved overall herbage CP concentration and reduced the NDF concentration of all three perennial ryegrass cultivars, compared to when they were grown as monocultures. This was due, in part, to the greater CP level and lower fibre concentration in white clover *per se* than perennial ryegrass as demonstrated in Chapter 3 and elsewhere (Andrews et al. 2007). Also, in Chapter 3, it was shown that the presence of white clover tended to increase herbage CP concentration and decrease herbage NDF concentration of accompanying perennial ryegrass. As for herbage WSC concentration and DOMD, the difference between white clover and perennial ryegrass was not large enough to alter overall herbage WSC concentration or DOMD of the mixture. Similarly, Enriquez-Hidalgo et al. (2014) found that compared to perennial ryegrass monoculture, perennial ryegrass-white clover mixtures tended to have a greater herbage CP concentration and a lower herbage NDF concentration with no significant differences in organic matter digestibility. However, there were significant interactions between the presence of white clover and the time of day for the WSC concentration and DOMD. This is because herbage WSC concentration and DOMD of perennial ryegrass vary more dramatically during the day than legumes (Orr et al. 1997; Morin et al. 2011). The interactions between the presence of white clover and regrowth stage for herbage chemical composition and digestibility were detected as well. Those interactions could be explained by the increasing white clover proportion in the mixtures over the regrowth period.

4.4.2 Perennial ryegrass cultivar

Perennial ryegrass cultivars differed in herbage WSC concentration, NDF concentration and DOMD, but not CP concentration. This result is in line with a previous study (Cosgrove et al. 2014), which showed that compared with the normal-sugar cultivars, the high-sugar cultivar was higher in WSC, lower in NDF but did not differ in the CP concentration. In agreement with the findings in Chapter 3, Prospect had a greater NDF concentration and a lower DOMD than AberMagic and Bealey which were similar in herbage NDF concentration and DOMD. AberMagic, a diploid cultivar bred for an elevated WSC concentration, expressed a greater WSC level throughout the experiment. Previous studies suggested that the high-sugar trait may not always be expressed (Cosgrove et al. 2007; Solomon et al. 2014) due to the cultivar × environment interaction (Cosgrove et al. 2007; Purcell et al. 2012). In this study, cultivar × management interaction could be another factor affecting the expression of high-sugar trait. At earlier regrowth stages, the differences in the WSC concentration among perennial ryegrass cultivars were relatively small. However, the WSC accumulated faster in AberMagic during pasture regrowth, creating a large difference among perennial ryegrass cultivars at the late regrowth stage. The cultivar ranking by CP and NDF concentration and DOMD changed during pasture regrowth. Similar interactions between perennial ryegrass cultivar and regrowth stage have been reported previously. Turner et al. (2015) found that the differences in the WSC concentration between high-sugar and normal-sugar cultivars were significantly greater at the 3-leaf stage than the 1.5-leaf stage. The presence of white clover and time of day did not interact with perennial ryegrass cultivar for herbage CP, WSC and NDF concentrations and DOMD. This suggested that white clover and harvest timing during the day did not affect the evaluation of the nutrient value of these three perennial ryegrass cultivars.

4.4.3 Regrowth stage

In keeping with previous studies (Fulkerson et al. 1998; Delagarde et al. 2000; Sun et al. 2010), herbage CP concentration and DOMD decreased, while WSC and NDF concentrations increased as regrowth period increased from 7 days to 28 days. During pasture regrowth, the ratio of plant cell content to cell wall decreases with an increasing concentration of NDF (Mambrini et al. 1994). Potentially, this could lead to a negative effect on DM intake and digestibility by requiring more time and energy for chewing and degradation of the herbage (Thornton & Minson 1972; Mambrini et al. 1994; Steg et al. 1994; Ayres et al. 1998). From a morphology point of view, the proportion of petiole and pseudostem relative to leaf blade above grazing level increases during pasture regrowth (Wilman & Asiegbu 1982; Bryant et al. 2012). As a result, overall herbage CP and WSC concentrations changed during pasture regrowth due to the distinct nutritive value of plant morphological parts (see Chapter 3). Additionally, WSC are mobilized and consumed in producing new plant tissues at earlier regrowth stages (Donaghy & Fulkerson 1998; Morvan-Bertrand et al. 2001) when energy supply from photosynthesis is inadequate (Morvan-Bertrand et al. 2001). This could also lead to a lower WSC concentration at the beginning of regrowth. In this study, herbage CP concentration decreased during pasture regrowth because of the dilution by the increasing WSC and NDF concentration, showing a negative relationship between WSC and CP concentrations (Reid & Strachan 1974). The CP concentrations of perennial ryegrass monocultures decreased more rapidly than the mixtures, because legumes showed less variation in the CP concentration during their regrowth compared to grasses (Gastal & Lemaire 2002).

4.4.4 Time of day

During the day, herbage WSC concentration increased dramatically in all cultivars. This result is consistent with previous findings. A greater WSC or nonstructural carbohydrates (NSC) concentration was observed in the herbage harvested at sunset, or in the evening, compared with herbage harvested at sunrise, or in the morning, in tall fescue (Holt & Hilst 1969; Fisher et al. 1999; Shewmaker et al. 2006), gamagrass (Sauvé et al. 2009), timothy (Pelletier et al. 2009), perennial ryegrass (Delagarde et al. 2000; Orr et al. 2001b), annual ryegrass (Gregorini et al. 2006) and alfalfa (Holt & Hilst 1969; Burns et al. 2005). This reflects the difference between plant photosynthesis and respiration (Orr et al. 2001b). Similar to previous results (Huntington & Burns 2007; Brito et al. 2008; Gregorini et al. 2009; Sauvé et al. 2009), the CP and NDF concentrations declined during the day with the improved digestibility. Delagarde et al. (2000) reported that nutrients, such as CP and NDF were passively diluted due to the accumulation of WSC during the day and the absolute amounts of herbage CP and NDF in the morning were similar to those in the evening (Delagarde et al. 2000; Rasmussen et al. 2009).

4.5 Conclusion

The white clover proportion in perennial ryegrass-white clover swards at grazing level increased during pasture regrowth. The presence of white clover significantly increased herbage CP concentration, reduced herbage NDF concentration and did not affect herbage DOMD without interactions with perennial ryegrass cultivar. Herbage WSC concentration increased during pasture regrowth and throughout the day, while NDF concentration increased during pasture regrowth but decreased throughout the day. CP concentration and DOMD showed an opposite pattern to WSC and NDF concentration, respectively. The interaction between perennial ryegrass cultivar and regrowth stage indicated a cultivar re-ranking by CP and NDF concentrations and DOMD during pasture regrowth. Therefore, pasture management had pronounced and consistent effects on the herbage nutrient value of perennial ryegrass pastures. The interactions between perennial ryegrass cultivar and regrowth stage will potentially confound the evaluation of perennial ryegrass cultivars.

Chapter 5

Rumen degradation of perennial ryegrass cultivars with different proportions of white clover

5.1 Introduction

Perennial ryegrass (*Lolium perenne* L.) is the most widely used temperate grass in north-west Europe, New Zealand, and in the temperate regions of Japan, South Africa and South America (Humphreys et al. 2010). Historically, improvements in productivity and persistence have been achieved through plant breeding and selection (Wilkins 1991). Previous perennial ryegrass breeding objectives have focused predominately on herbage dry matter (DM) yield, including annual DM yield and seasonal DM yield distribution (i.e. heading date), resistance to diseases and pest and persistence (Stewart & Hayes 2011). Recently, increased emphasis has been placed on feeding value, which combines herbage nutritive value and animal voluntary intake (Chapman et al. 2014).

The rapid herbage degradability in the rumen is a desirable trait as it could lead to increased voluntary DM intake by grazing animals because of the less retention time in the rumen (Sun et al. 2010). Sun et al. (2012) found considerable genotypic variation in rumen DM degradation rate among 77 perennial ryegrass accessions, indicating an opportunity to select for faster degrading cultivars. In addition to DM disappearance rate, nitrogen (N)/energy balance and synchrony are also critical in the rumen for effective microbial synthesis. If the energy supply is insufficient or asynchronous, rumen-degraded protein is absorbed as ammonia via the rumen wall, detoxified into urea in the liver and mostly excreted in urine (Tamminga 1996). Consequently, an unbalance or asynchrony of N/energy in the rumen may result in low nitrogen-use efficiency (NUE) and environmental pollutions, particularly as nitrate leaching and greenhouse gases emissions (Di & Cameron 2002). Therefore, increasing water-soluble carbohydrates (WSC) as a rapidly fermentable energy source could hypothetically improve the balance of N and energy supply in the rumen. Although Tas et al. (2006b) found little genetic variations in rumen degradation among eight diploid perennial ryegrass cultivars, more information is needed regarding tetraploid cultivars and high-sugar cultivars.

To maintain sustainable, low cost and low input agricultural system, white clover (*Trifolium repens* L.) is usually included in perennial ryegrass pastures (Peoples & Baldock 2001). It has a superior nutritive value and is palatable to grazing animals (Frame et al. 1998). On the other hand, N fixation results in a high N concentration in white clover herbage, which may lead to a poor N/energy balance and synchrony in the rumen (Belanche et al. 2013). Jaurena et al. (2005) suggested that NUE in the rumen will be potentially improved when an adequate amount of carbohydrates is supplied from, for example, perennial ryegrass (Moorby et al. 2009). White clover is likely to stabilise at around 20% of herbage DM yield in a well-maintained perennial ryegrass-white clover mixed pasture (Andrews et al. 2007), and the proportion of white clover may be greater in the diet due to the dietary selection (Cosgrove & Edwards 2007). However, little information is available on the rumen degradation characteristics of perennial ryegrass-white clover mixtures. Min et al. (2000) reported that diet affected rumen microorganisms, leading to changes in rumen degradation. Additionally, secondary metabolites, such as condensed tannins in plants may cause digestive interactions when animals were fed a mixed diet, resulting in misleading predictions of the mixtures from individual dietary components (Niderkorn et al. 2011).

The aim of this study was to compare rumen degradation characteristics among three perennial ryegrass cultivars, including DM disappearance rate and N/energy balance and synchrony in the rumen. Rumen degradations of perennial ryegrass-white clover mixtures with different white clover proportions were also evaluated. The findings of this study could thus provide information for plant breeding and pasture management, and a better understanding of the rumen function.

5.2 Materials and Methods

Two experiments were conducted at Lincoln University Research Dairy Farm, Canterbury, New Zealand (43°38'S, 172°27'E, 12 m above sea level) in March and April 2015, using four rumen-fistulated Friesian × Jersey cows. All procedures were approved by the Lincoln University Animal Ethics Committee (AEC 607).

Table 5.1 DM percentage (%) and chemical composition (g/kg DM) of original herbage samples in experiment I and II.

Treatment	DM	OM	CP	WSC	NDF
Experiment I: Perennial ryegrass cultivars and white clover					
AberMagic	22.5	920	170	304	451
Bealey	23.5	922	164	268	444
Prospect	20.8	913	179	277	457
White clover	16.6	905	296	121	276
Experiment II: White clover proportion†					
0%	19.3	894	203	-	-
16.7%	18.6	895	212	-	-
33.3%	17.6	893	231	-	-
100%	14.5	894	324	-	-

†White clover proportion was on a fresh weight basis

5.2.1 Experimental design

In each experiment, four rumen-fistulated lactating cows were used in a 4 × 4 Latin square design with four treatments and four periods. In experiment I, *in sacco* degradation characteristics of three perennial ryegrass cultivars (AberMagic AR1, the high-sugar diploid cultivar; Bealey NEA2, the tetraploid cultivar; Prospect AR37, the normal-sugar diploid cultivar) and white clover (Kopu II) were investigated (Table 5.1). These three perennial ryegrass cultivars were selected out of the eight cultivars in Chapter 3, representing a tetraploid (Bealey) vs. diploid cultivars (AberMagic and Prospect) comparison, and a high-sugar (AberMagic) vs. normal-sugar cultivars (Prospect) comparison. In experiment 2, the evaluation was conducted among four mixtures of perennial ryegrass and white clover with different white clover proportions (Table 5.1).

5.2.2 Sampling and preparation

In experiment I, perennial ryegrass cultivars were harvested from their monocultures under a high N fertiliser application rate (325 kg N/ha) as described in 3.2.1 and 3.2.2 in October 2014. White clover (Kopu II) were harvested from its monoculture plots in November 2014 (Rossi 2016). Herbage was harvested by a Haldrup forage harvester between 1000 and 1200 h at grazing level (5.5 cm above ground level). About 3.5 kg of fresh herbage of each treatment were taken. Subsamples of approximately 40 g fresh weight (FW) were cut at a length of 1 cm with a guillotine and then placed into numbered nylon bags (pore size: 50 microns). Bags were closed with a nylon string creating a loop for attaching (Figure 5.1a). Sixty duplicate nylon bags of each treatment were prepared and stored in the freezer at -20°C until needed.

In experiment II, perennial ryegrass (AberMagic) and white clover (Kopu II) were harvested the same way as experiment I in February and March 2015, respectively. Perennial ryegrass and white clover herbage were mixed into four treatments on a FW basis: 100% perennial ryegrass, 83.3% perennial ryegrass + 16.7% white clover, 66.7% perennial ryegrass + 33.3% white clover and 100% white clover. Sixty duplicate nylon bags of each combination were prepared as described above with approximately 30 g FW per bag (because less herbage material was available in experiment II).

Another subsample of about 50 g FW from each treatment in both experiment I and II was weighed and oven-dried at 60°C for 48 h to obtain herbage DM percentage. The oven-dried original samples were further analysed for chemical composition.

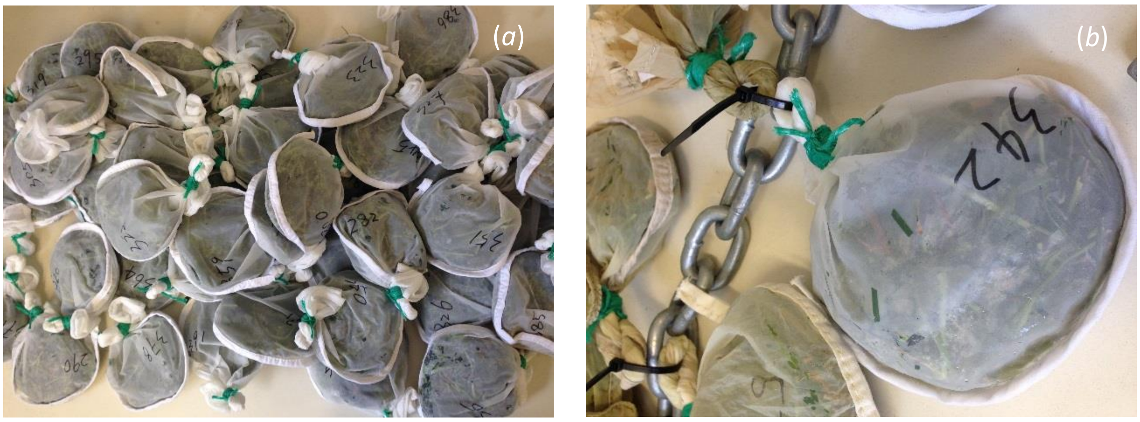


Figure 5.1 Nylon bag (*a*) and chain (*b*) preparations.

5.2.3 Rumen incubation

Four lactating rumen-fistulated cows were used for incubations in four periods for each experiment. Treatments were allocated to a different cow in each period. Before and during the trial, the cows continuously grazed on a perennial ryegrass-white clover pasture and were milked twice a day at 0730 h in the morning and 1400 h in the afternoon. All rumen incubation commenced after morning milking at around 0800 h. Before incubation, frozen nylon bags with samples were warmed in a water bath (40°C) for 20 min as suggested by Sun and Waghorn (2012). Fifteen duplicated bags from one treatment were then attached to a chain (Figure 5.1b). Four chains were prepared and allocated to different cows in each period. Two parallel bags were retrieved before they were put in the rumen (0 h bags) and after rumen incubation for approximately 3, 6, 9, 12 and 24 h. Three parallel bags were retrieved after 48 h because of the small amount of residual herbage. Actual times of bag retrieving were recorded for the statistical analysis. Retrieved bags were put into ice water for 5 min and then washed with tap water to clean the exterior before keeping in the freezer. All bags were thawed in water at 4°C, washed in a washing machine together, oven-dried at 60°C for 48 h and weighed. After residuals were removed for chemical analysis (Figure 5.2), nylon bags were washed, oven-dried and weighed again to calculate the residual DM weight.

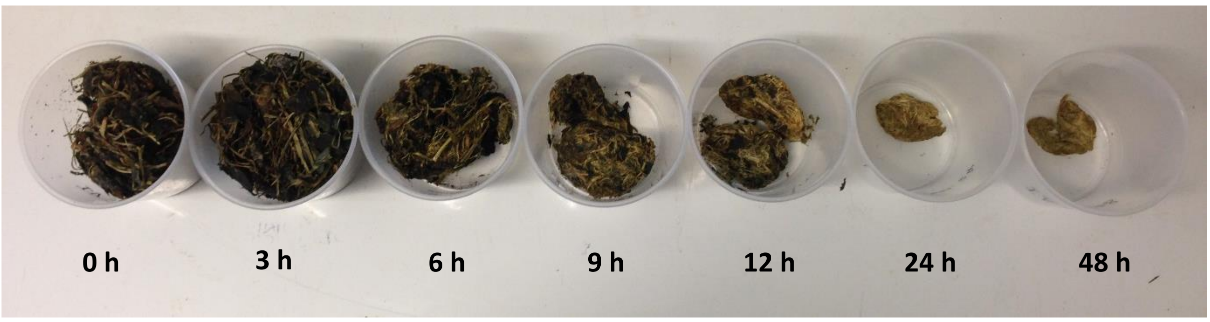


Figure 5.2 Nylon bag residuals after rumen incubation.

5.2.4 Chemical composition analysis

Oven-dried original subsamples and nylon bag residuals (bulked from parallel bags) were analysed for chemical composition. Samples were ground through a 1-mm sieve and then analysed for N concentration by Elementar (Vorio MAX CN) and organic matter (OM) concentration (ashed at 500°C for 2 h). The crude protein (CP) concentration was calculated by multiplying N concentration by 6.25. Original herbage subsamples in experiment I were analysed by near infrared reflectance spectroscopy (NIRS Systems 5000, Foss, Maryland, USA) for the concentrations of WSC and neutral detergent fibre (NDF). NIRS calibrations were previously developed for WSC (MAFF 1986) and NDF (van Soest et al. 1991). Assays for NDF excluded sodium sulphite or α-amylase and NDF predictions were inclusive of residual ash (Bryant et al. 2012).

5.2.5 Calculations

Rumen degradability

The proportion of DM, OM and CP loss from nylon bags during incubation were fitted to the following equation as described by Ørskov and McDonald (1979) using SigmaPlot (version 13.0).

$$P = a + b(1 - e^{-ct}),$$

where P is degradability of DM, OM or CP, a represents the soluble fraction, b is the insoluble-degradable fraction, c is the fractional degradation rate of fraction b (/h) and t is the incubation time (h).

Potential and effective degradability

Potential degradability was calculated as '$a + b$' and thus undegradable fraction was '$1 - (a + b)$'. Due to the limited rumen retention time, effective degradability was calculated as '$a + bc/(c + k)$' (Ørskov & McDonald 1979), representing the portion of DM, OM or CP available for rumen microbes. Fractional passage rate, k was assumed to be 0.06/h for typical dairy cows fed perennial ryegrass (van Vuuren et al. 1991).

As suggested by AFRC (1993), the efficiency of quickly degradable protein (QDP, a fraction) is 0.8, which is the proportion of QDP actually captured by rumen microbes. Slowly degradable protein (SDP, b fraction) cannot be completely degraded in rumen because of the rumen outflow. Therefore, effective rumen degradable protein (ERDP) was defined as follow:

QDP (g/kg DM) = a × CP,

SDP (g/kg DM) = $bc/(c + k)$ × CP,

ERDP (g/kg DM) = 0.8 × QDP + SDP.

N/energy balance and synchrony

In order to plot all the data in the same scale, herbage samples in every bag were standardised to 10 g OM per bag for uniformity. N release rate relative to OM disappearance from bags were fitted to a cubic polynomial curve using SigmaPlot 13.0 (Table 5.2). The balance was expressed as N release/OM disappearance, while synchrony was calculated as the derivative of the curve. N/energy balance and synchrony were compared with the standard value, 20.3 g N/kg OM, recommended by Czerkawski (1978).

Table 5.2 Parameters in cubic polynomial regression equations ($y = y_0 + ax + bx^2 + cx^3$) demonstrating N release (*y*) and OM disappearance (*x*) during the incubations in experiment I and II.

Treatment	Parameters				R^2	*P* value
	y_0	a	b	c		
Experiment I						
AberMagic	-6.91×10^{-5}	-1.62×10^{-2}	1.37×10^{-2}	-9.53×10^{-4}	0.996	< 0.01
Bealey	8.66×10^{-2}	-7.35×10^{-2}	2.24×10^{-2}	-1.33×10^{-3}	0.996	< 0.01
Prospect	-2.23×10^{-2}	2.48×10^{-2}	5.27×10^{-3}	-4.68×10^{-4}	0.986	< 0.01
White clover	-1.21×10^{-1}	1.03×10^{-1}	-5.45×10^{-3}	1.77×10^{-4}	0.997	< 0.01
Experiment II						
0%	-7.05×10^{-2}	4.42×10^{-2}	5.51×10^{-3}	-5.93×10^{-4}	0.995	< 0.01
16.7%	-3.78×10^{-2}	2.55×10^{-2}	8.86×10^{-3}	-7.59×10^{-4}	0.996	< 0.01
33.3%	-6.22×10^{-2}	3.52×10^{-2}	8.75×10^{-3}	-7.81×10^{-4}	0.997	< 0.01
100%	-5.29×10^{-2}	5.90×10^{-2}	2.85×10^{-3}	-2.24×10^{-4}	0.998	< 0.01

5.2.6 Statistical analysis

For both experiment I and II, degradation parameters (*a*, *b* and *c*), potential degradability, effective degradability of DM, OM and CP, and ERDP concentration were analysed in a general linear model by SPSS (version 22.0):

$$y = \mu + C_i + P_j + T_k + \varepsilon_{ijk},$$

where y is the dependent variable, μ is the overall mean, C_i (i = 1, 2, 3, 4) is the effect of cows, P_j (j = 1, 2, 3, 4) is the effect of period, and T_k (k = 1, 2, 3, 4) is the effect of treatment. ε_{ijk} is the residual error.

In experiment II, a simple linear regression was performed on SPSS (version 22.0) between the proportion of white clover in the mixtures and their degradation parameters (*a*, *b* and *c*), potential degradability, effective degradability of DM, OM and CP, and ERDP concentration.

5.3 Results

5.3.1 Perennial ryegrass cultivars and white clover

In experiment I, white clover and Bealey had a greater soluble fraction, *a*, and degradation rate, *c*, of DM and OM than AberMagic and Prospect ($P < 0.05$, Table 5.3). This, in turn, led to greater DM and OM effective degradabilities of Bealey than AberMagic and Prospect ($P < 0.05$). However, potential degradabilities, '*a* + *b*', of DM and OM were similar between perennial ryegrass and white clover and among perennial ryegrass cultivars ($P < 0.05$). In terms of CP degradation, white clover had a greater *a*, and a lower *b*, compared to perennial ryegrass ($P < 0.05$, Table 5.3), which resulted in a greater effective degradability of white clover than perennial ryegrass (68.9% vs. 59.9%, $P < 0.05$). A greater ERDP concentration was found in white clover (188.9 g/kg DM, $P < 0.01$), because of the combined effect of white clover's greater effective degradability and higher CP concentration (269 g/kg DM). Among perennial ryegrass cultivars, Prospect had a greater CP soluble fraction, *a*, than AberMagic and Bealey (0.132 vs. 0.082 and 0.062, $P < 0.05$).

Regarding N/energy synchronisation, white clover had a greater N release rate relative to OM disappearance in both soluble and insoluble-degradable fractions than all perennial ryegrass cultivars (Figure 5.3). Due to the low soluble fraction of CP, the N release rates of were less than 20.3 g N/kg OM in the soluble fraction of AberMagic and Bealey (Figure 5.3). Bealey had the lowest accumulative N release rate relative to OM disappearance (28.9 g N/kg OM at the effective degradability).

Table 5.3 Rumen degradation characteristics of perennial ryegrass cultivars and white clover in experiment I.

		AberMagic	Bealey	Prospect	White clover	SEM†	*P* value
DM	Degradation parameters						
	a	0.276^{b}	0.305^{a}	0.234^{c}	0.310^{a}	0.0052	< 0.01
	b	0.641	0.621	0.669	0.629	0.0153	0.24
	c	0.066^{b}	0.081^{a}	0.063^{b}	0.081^{a}	0.0029	< 0.01
	Potential degradability (%)	91.7	92.6	90.3	93.9	1.19	0.27
	Effective degradability (%)	61.1^{b}	65.9^{a}	57.3^{c}	67.0^{a}	0.58	< 0.01
OM	Degradation parameters						
	a	0.246^{b}	0.280^{a}	0.202^{c}	0.282^{a}	0.0056	< 0.01
	b	0.675	0.647	0.701	0.651	0.0151	0.14
	c	0.065^{b}	0.080^{a}	0.062^{b}	0.081^{a}	0.0028	< 0.01
	Potential degradability (%)	92.1	92.8	90.4	93.4	1.15	0.36
	Effective degradability (%)	59.5^{b}	64.8^{a}	55.6^{a}	65.5^{a}	0.59	< 0.01
CP	Degradation parameters						
	a	0.082^{c}	0.062^{c}	0.132^{b}	0.261^{a}	0.0133	< 0.01
	b	0.855^{ab}	0.894^{a}	0.798^{b}	0.706^{c}	0.0180	< 0.01
	c	0.087	0.099	0.084	0.094	0.0054	0.32
	Potential degradability (%)	93.7	95.6	93.0	96.7	0.92	0.09
	Effective degradability (%)	58.9^{b}	61.5^{b}	59.3^{b}	68.9^{a}	0.94	< 0.01
	ERDP (g/kg DM)	97.0^{b}	99.1^{b}	101.6^{b}	188.9^{a}	2.02	< 0.01

†SEM, standard error of the mean
Means followed by different superscripts are significantly different by Duncan's multiple range test (P < 0.05)

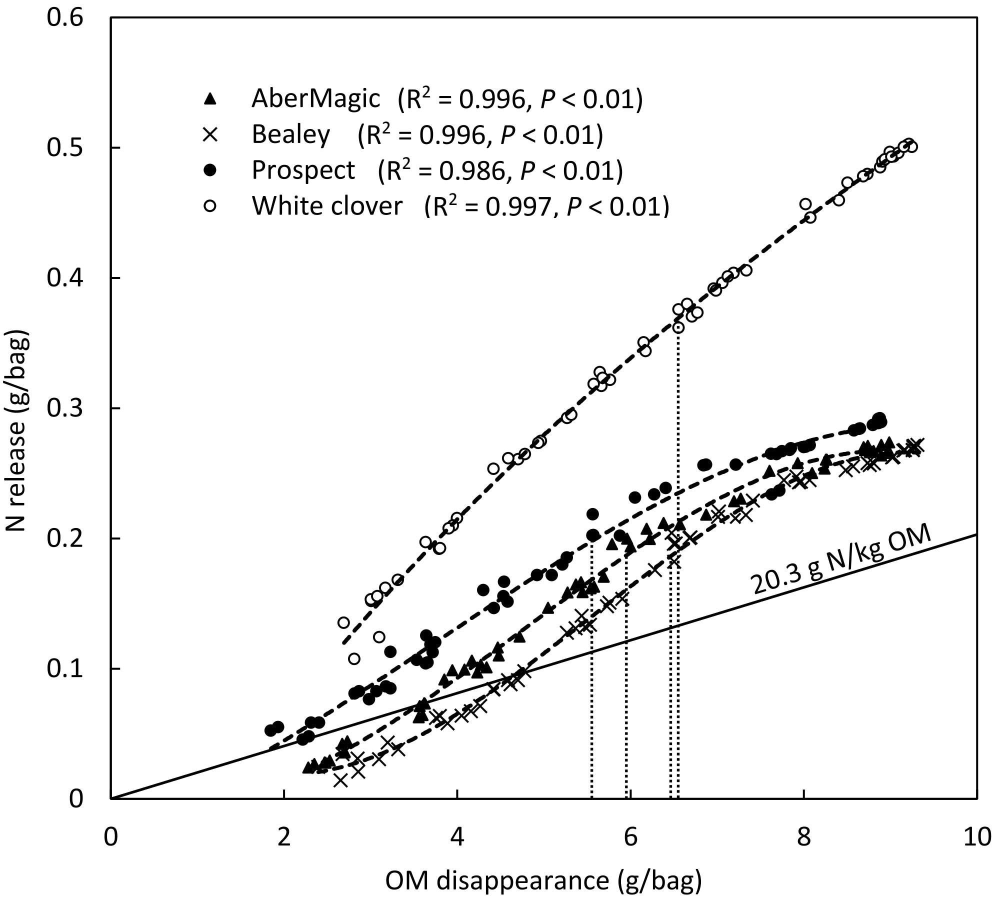


Figure 5.3 N released and OM disappearance from nylon bags during the incubations of perennial ryegrass cultivars and white clover in experiment I. The initial amount of OM was uniformed to 10 g. The recommended efficiency of synthesis of microbial N per OM truly digested in the rumen is 20.3 g N/kg OM (Czerkawski 1978). Vertical dash lines denote the effective degradability of corresponding treatments.

5.3.2 Mixtures of perennial ryegrass and white clover

In experiment II, perennial ryegrass and white clover had a similar soluble fraction, *a*, and insoluble-degradable fraction, *b*, of DM and OM ($P > 0.05$, Table 5.4). Therefore, there was no difference in *a* and *b* among their mixtures ($P > 0.05$). However, potential degradability, '*a* + *b*' was greater in pure white clover and pure perennial ryegrass than their mixtures ($P < 0.01$, Table 5.4). Degradation rates, *c*, of DM and OM were greater in white clover than perennial ryegrass ($P < 0.01$). Consequently, effective degradability of the mixtures increased linearly with white clover proportion (DM effective degradability from 57.4% to 75.8%, $P < 0.01$; OM effective degradability from 54.7% to 74.5%, $P < 0.01$). In terms of CP degradation, white clover had a greater soluble fraction, *a* (0.167), and fractional degradation rate, *c* (0.170/h). However, the regressions between white clover proportion and *a* and *b* of DM, OM and CP were not significant ($P > 0.05$). In mixtures, the fractional degradation rate, *c*, effective degradability of CP and ERDP concentration increased proportionally as white clover proportion increased in the mixture ($P < 0.01$).

Similar to experiment I, N release rate relative to OM disappearance was greater in pure white clover than pure perennial ryegrass in both soluble and insoluble-degradable fractions (Figure 5.4). Both instantaneous and accumulative N release rate in insoluble-degradable fractions increased as white clover proportion increased in the mixtures with the values always greater than the standard value, 20.3 g N/kg OM (Figure 5.4).

Table 5.4 Rumen degradation characteristics of perennial ryegrass and white clover mixtures in experiment II.

		White clover proportion†				SEM‡	*P* value	
		0%	16.7%	33.3%	100%		Treatment	Linear regression
DM	Degradation parameters							
	a	0.257	0.262	0.253	0.277	0.0074	0.22	0.18
	b	0.670	0.647	0.647	0.671	0.0086	0.15	0.60
	c	0.055[b]	0.067[b]	0.084[b]	0.159[a]	0.0126	< 0.01	< 0.01
	Potential degradability (%)	92.8[b]	90.9[c]	90.0[c]	94.8[a]	0.47	< 0.01	0.07
	Effective degradability (%)	57.4[c]	60.3[bc]	62.8[b]	75.8[a]	1.27	< 0.01	< 0.01
OM	Degradation parameters							
	a	0.210	0.223	0.207	0.236	0.0084	0.17	0.15
	b	0.720	0.687	0.690	0.708	0.0099	0.16	0.96
	c	0.054[b]	0.066[b]	0.083[b]	0.161[a]	0.0128	0.02	< 0.01
	Potential degradability (%)	93.0[a]	91.0[b]	89.8[b]	93.0[a]	0.50	< 0.01	0.17
	Effective degradability (%)	54.7[c]	58.3[bc]	60.6[b]	74.5[a]	1.37	< 0.01	< 0.01
CP	Degradation parameters							
	a	0.113[bc]	0.143[ab]	0.093[c]	0.167[a]	0.0088	< 0.01	0.10
	b	0.859[ab]	0.819[b]	0.873[a]	0.817[b]	0.0122	0.04	0.32
	c	0.082[b]	0.092[b]	0.118[b]	0.170[a]	0.0131	0.01	< 0.01
	Potential degradability (%)	97.2	96.3	96.6	98.4	0.508	0.09	0.05
	Effective degradability (%)	60.5[c]	64.0[bc]	67.0[b]	76.6[a]	1.34	< 0.01	< 0.01
	ERDP (g/kg DM)	118.4[d]	129.5[c]	150.7[b]	237.1[a]	3.20	< 0.01	< 0.01

†White clover proportion was on a fresh weight basis
‡SEM, standard error of the mean
Means followed by different superscripts are significantly different by Duncan's multiple range test ($P < 0.05$)

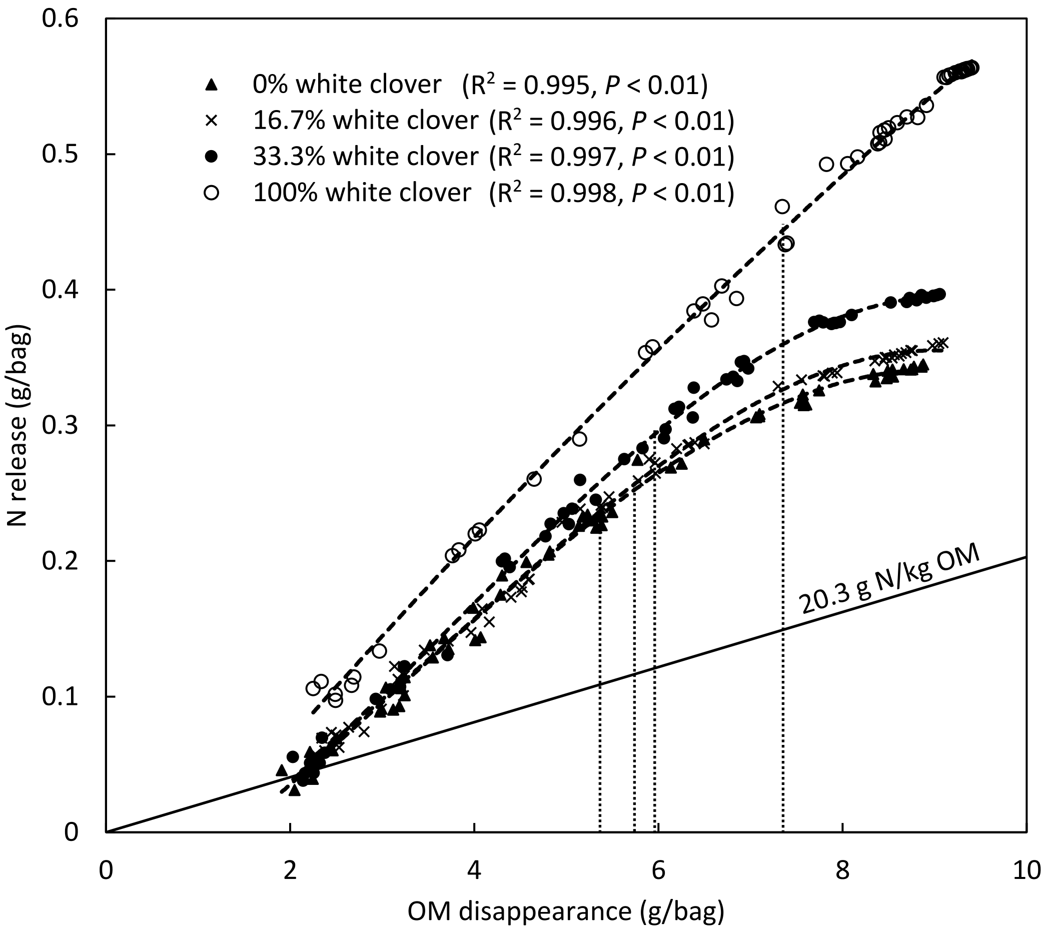


Figure 5.4 N release and OM disappearance from nylon bags during incubations of perennial ryegrass and white clover mixtures in experiment II. The initial amount of OM was uniformed to 10 g. The recommended efficiency of synthesis of microbial N per OM truly digested in the rumen is 20.3 g N/kg OM (Czerkawski 1978). Vertical dash lines denote the effective degradability of corresponding treatments.

5.4 Discussion

5.4.1 Methodology and calculations

In this study, the soluble fraction, *a*, of DM, OM and CP from chopped herbage samples were lower than the values reported previously using minced herbage samples (Hoffman et al. 1993; Chaves et al. 2006b; Sun & Waghorn 2012). Dhanoa et al. (1999) stated that small particles, at least partly, could escape from the nylon bag by washing at 0 h, leading to an overestimate of the soluble fraction, *a*, from ground or minced samples. In this study, it is likely that freezing and thawing induced disruption of plant structures, which may offset the leaching reduction by insufficient physical mastication from chopping (López et al. 1995; Hristov 1998).

Previously, synchrony indexes have been developed to reflect N release relative to OM disappearance (Sinclair et al. 1993; Verbič et al. 1999). However, there are some limitations of these approaches. Firstly, previous synchrony index calculations were based on arithmetic means throughout the whole degradation process. However, it is clear that the majority of OM disappears at the early stage of degradation rather than the later stages, which means the earlier stage of degradation should contribute more to the synchrony index. Secondly, many previous synchrony index calculations took the rumen degradation after the effective degradability into account. However, that should be excluded because the feed will theoretically pass through the rumen when they reach the effective degradability. To overcome this, Tas et al. (2006b) calculated the ratio between effectively degraded N to OM. However, this is an accumulative N/energy balance, which does not describe the N/energy synchrony during the degradation process.

To overcome these limitations, in this study, degraded N and OM from individual nylon bags were plotted after being uniformed by standarising OM per bag. Therefore, the starting point of the curve demonstrates the N/energy balance in the soluble fraction; the derivative of the curve indicates the N/energy synchrony during the degradation process (comparing the slop to the standard value). Thus, the ratio of N release to OM disappearance at effective degradability shows the accumulative N/energy balance in the rumen.

5.4.2 Rumen degradation of perennial ryegrass cultivars

In this study, DM and OM degradation rates of the tetraploid cultivar, Bealey, was greater than the diploid cultivars, AberMagic and Prospect. This was probably because the tetraploid cultivar have a higher ratio of cell content to the cell wall (Stewart & Hayes 2011) that mainly consist of fibres and limit nutrient release (Chaves et al. 2006b). Miller et al. (2001) suggested that the high-sugar diploid cultivar had a greater DM disappearance rate than the control diploid counterpart (0.124 vs. 0.058/h). In this study, the similarity of the NDF concentrations of AberMagic and Prospect (Chapter

3) led to similar DM and OM degradation rate, which is in agreement with previous research (Taweel et al. 2005a; Tas et al. 2006b). DM and OM potential degradabilities (i.e. undegradable fractions) were similar among perennial ryegrass cultivars, while effective degradability was greater in Bealey due to its greater degradation rate. In conclusion, the tetraploid cultivar, Bealey, met the breeding criteria of DM degradation suggested by Sun et al. (2012), which includes a greater proportion of soluble fraction, *a*, and a faster insoluble-degradable fraction, *b*.

Prospect had a greater CP concentration and soluble fraction, *a*, than Bealey and AberMagic, leading to a greater N/energy ratio in its soluble fraction. Because the soluble fraction, *a*, of CP was less than that of DM and OM, perennial ryegrass did not have a higher proportion of soluble CP than white clover as expected from previous studies (Bryant et al. 2012), and generally expressed a low N/energy ratio in its soluble fraction. This result is in agreement with a previous study, in which fresh perennial ryegrass was investigated for OM and CP degradation characteristics (van Vuuren et al. 1991).

The N release rates relative to OM disappearance that was defined as the slope in Figure 5.3 were greater than the standard value of 20.3 g N/kg OM (Czerkawski 1978) in diploid cultivars, AberMagic and Prospect. The accumulative N release rate relative to OM disappearance was lower in the tetraploid cultivar, Bealey, indicating the best N/energy balance among these three perennial ryegrass cultivars. This finding could be explained by the low herbage CP concentration and the greater OM degradation rate of Bealey (Chen et al. 2016) when CP degradation rates were similar among cultivars.

5.4.3 Rumen degradation of white clover and perennial ryegrass mixtures

In agreement with a previous study (Moharrery et al. 2009), results from both experiment I and II showed that white clover had a greater soluble fraction and a faster degradation rate of DM than perennial ryegrass. Reticulum venations in white clover with lower hemicellulose and cellulose contents led to the faster rates of particle breakdown (Steg et al. 1994; Wilman et al. 1996) and greater effective degradabilities than the grass species with parallel venations (Fulkerson et al. 1998; Williams et al. 2005a).

DM and OM potential degradability of perennial ryegrass-white clover mixtures did not increase linearly as the proportion of white clover increased. This digestive interaction between perennial ryegrass and white clover made potential degradabilities lower in their mixtures than either individual component. In previous studies, the presence of condensed tannins, a plant secondary compound, has been the main reason for many digestive interactions of CP, due to its ability to inhibit proteolytic enzymes (Min et al. 2003; Burggraaf et al. 2008). However, white clover leaves and perennial ryegrass have been found to be free of condensed tannin (Burggraaf et al. 2008). Additionally, in this study, perennial ryegrass-white clover mixtures had greater undegradable fractions of DM, OM but not CP. The potential reason for this interaction thus could be the shift in the bacterial community caused by the different diets (Belanche et al. 2013). Nevertheless, this interaction only happened for potential degradababilities, but not for effective degradabilities. Given that the feed will theoretically pass through the rumen when they reach their effective degradabilities, this digestive interaction could be ignored in cultivar evaluations.

In this study, N release rates relative to OM disappearance of the perennial ryegrass-white clover mixtures were around 60 g N/kg OM, much greater than 20.3 g N/kg OM recommended by Czerkawski (1978) for dairy cows. Further, N release rates relative to OM disappearance increased proportionally as white clover was added to the diet. White clover is characterised by a high CP concentration with a relatively low WSC concentration (Evans et al. 1996; Chen et al. 2016). The excess N leads to the low efficiency of utilisation for animals fed clover (van Dorland et al. 2006). Theoretically, supplying an adequate amount of carbohydrates to white clover may be a strategy to improve the efficiency of N utilisation in the rumen, such as perennial ryegrass cultivars with an elevated WSC concentration (Moorby et al. 2009). However, in this study, N fertiliser was applied intensively (325 kg N/ha/year) to maintain a high annual herbage DM yield, thus leading to a particularly high CP concentration in perennial ryegrass (203 g/kg DM), which is common in New Zealand rotational grazing systems (Wilkins et al. 2000). Therefore, even though perennial ryegrass had a more synchronised N and energy availability than white clover (van Dorland et al. 2006), N release rates relative to OM disappearance of the mixtures were still greater than the recommended value. The results also showed that perennial ryegrass monoculture and perennial ryegrass-white clover mixtures with a low proportion of white clover (16.7% of FW) were similar in N/energy synchrony before they reached their effective degradabilities in the rumen. This means that including white clover will exert a negative but limited influence on N/energy balance and synchrony in a well-managed mixture pasture which generally contains about 20% white clover (on a DM basis) (Andrews et al. 2007). Nevertheless, white clover would still benefit the environment, production and profitability by reducing the reliance on inorganic N fertiliser and enhancing voluntary DM intake of grazing animals (van Dorland et al. 2006).

5.5 Conclusion

The tetraploid cultivar, Bealey, had a greater soluble fraction, *a*, and a faster insoluble-degradable fraction, *b*, of both DM and OM. In terms of CP degradation, the normal-sugar diploid cultivar, Prospect had a greater soluble fraction, *a*, than the high-sugar diploid cultivar, AberMagic, and the tetraploid cultivar, Bealey. Bealey had the lowest accumulative and instantaneous N release rates relative to OM disappearance, indicating the best N/energy balance and synchrony among these three cultivars. CP effective degradability and ERDP concentration of perennial ryegrass-white clover mixtures increased proportionally as white clover proportion increased in the mixtures. White clover exerted negative influences on N/energy balance and synchrony in their mixtures. However, the impact would be limited when the white clover proportion was low.

Chapter 6

Dietary preference and selection of dairy cows for perennial ryegrass cultivars growing with and without white clover

6.1 Introduction

An understanding of the dietary preference and selection of grazing animals is important for the efficient utilisation of pasture (Rutter 2006). It is also useful for both farmers and breeders to improve animal voluntary dry matter (DM) intake and performance (Edwards et al. 2008). Preference and selection are exhibited not only for different species (Francis et al. 2006; van Dorland et al. 2006) but also for cultivars of the same species (Shewmaker et al. 2006; Solomon et al. 2014). 'Selection' is a function of 'preference' that represents what animals would like to eat (Rutter 2006) and the opportunity for selecting under environmental constraints (Hodgson 1979).

Dietary preference and selection of dairy cows, beef cattle and sheep have been evaluated for perennial ryegrass (*Lolium perenne* L.) cultivars (Roegiers et al. 1988; Smit et al. 2006). Most of these studies were carried out using pure perennial ryegrass pastures rather than perennial ryegrass-white clover mixtures. Growing white clover (*Trifolium repens* L.) with perennial ryegrass is a common practice in temperate pastures (Peoples & Baldock 2001). Due to the ecological complexity of a mixture pasture, each botanical component may only explain a portion of the potential animal performance (Lee et al. 2012). Also, the interaction between pasture components may either diminish or enhance the differences in animal performance from different perennial ryegrass cultivars. Frame and Boyd (1986) suggested that perennial ryegrass cultivars with different sward structures may potentially support different amounts of white clover in the swards (Frame & Boyd 1986). A small change in white clover proportion could lead to a disproportionate effect on animal production and outweigh the perennial ryegrass cultivar effect (Hyslop et al. 2000). Hypothetically, including white clover in perennial ryegrass pastures might lead to changes in dietary preference and selection for perennial ryegrass cultivars.

The objectives of this study were (1) to measure the sward structure, morphology, nutritive value, dietary preference and selection for eight perennial ryegrass cultivars growing with and without white clover, (2) to investigate the effect of white clover on dietary preference and selection for perennial ryegrass cultivars and (3) to define what herbage characteristics contributed to dietary preference and selection.

6.2 Materials and methods

6.2.1 Experimental design

This study was conducted within the experiment described in 3.2.1 and 3.2.2. It was a split-plot design with four blocks under the high nitrogen (N) fertiliser application rate (325 kg N/ha). The main-plot factor was the presence of white clover, the sub-plot factor was perennial ryegrass cultivar (Figure 3.1). Each main-plot contained eight perennial ryegrass cultivars in adjacent sub-plots (Table 3.1). The white clover sown in the experiment was a 50:50 mixture of cultivar Kopu II and Tribute (sown at 4 kg/ha).

6.2.2 Animal management

Three grazing experiments were conducted at three phenological stages: pre-heading vegetative (early spring, October 2014), reproductive (late spring, November 2014) and post-flowering vegetative (autumn, May 2014). In each grazing experiment, eighty Friesian × Jersey crossbred dairy cows were randomly divided into eight groups of ten cows. Groups were not balanced according to weight, age or milk yield. Each group was allocated to one of the eight main-plots having free choices among eight adjacent perennial ryegrass cultivars after morning milking (around 0730 h). Cows grazed the paddocks to the target residual height, 4 to 5 cm, over 6 to 8 h depending on the herbage availability. Cows had *ad libitum* access to water.

6.2.3 Sward measurements

Herbage mass was measured before and after grazing by cutting three 0.2 m^2 quadrats in each sub-plot to ground level using hand shears, respectively. Quadrats were randomly located with an avoidance of 3 m end of each sub-plot and the areas affected by urine and dung patches. A subsample of approximately 50 g fresh weight was taken from each quadrat sample for botanical composition and morphological measurements. Perennial ryegrass, white clover (in treatments where white clover was present), dead material and weeds were sorted by hand. Ten intact perennial ryegrass tillers and white clover leaves were selected randomly. The length of the newest fully expanded grass leaf blades (from ligule to tip) and white clover petiole were recorded. All herbage samples, fractions and tillers were oven-dried at 60°C for 48 h and weighed so that herbage mass, botanic composition and tiller mass could be calculated on a DM basis. Tiller density was calculated based on the herbage mass, the proportion of perennial ryegrass and the average tiller mass.

In order to monitor the herbage disappearance during grazing, 30 measurements of perennial ryegrass sward surface height (SSH) were recorded in each sub-plot after 0, 1, 2, 4 and 6 or 8 h of grazing using a sward stick (Jenquip, New Zealand, Figure 6.1a). In order to reflect the surface height, measurements were taken based on the natural position of the leaf (Figure 6.1b) and did not include reproductive components, i.e. inflorescences.

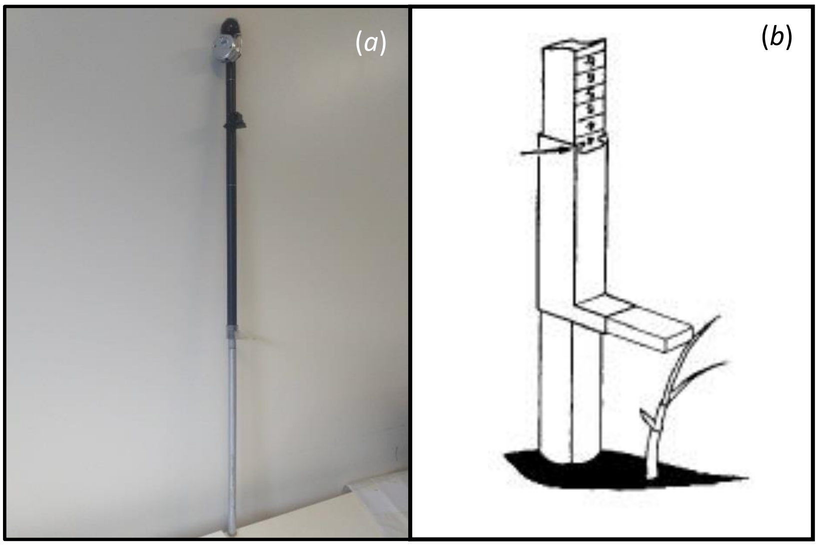


Figure 6.1 Sward stick (*a*) and how measurements were taken (*b*).

6.2.4 Chemical composition and digestibility analysis

Additional herbage samples of 150 g fresh weight were cut to ground level from at least six locations in each sub-plot. Fresh samples were placed in a chilly bin immediately, transferred to the laboratory as soon as possible. Samples were oven-dried at 60°C for 48 h, weighed and ground through a 1-mm sieve. Ground samples were analysed by near infrared reflectance spectroscopy (NIRS Systems 5000, Foss, Maryland, USA) for chemical composition, including the concentrations of organic matter (OM), crude protein (CP), acid detergent fibre (ADF), neutral detergent fibre (NDF) and water-soluble carbohydrates (WSC) and organic matter digestibility in dry matter (DOMD). NIRS calibrations were previously derived on perennial ryegrass samples (Jones & Hayward 1975; MAFF 1986; AOAC 1990; van Soest et al. 1991). Assays for NDF excluded sodium sulphite or α-amylase and both ADF and NDF predictions were inclusive of residual ash (Bryant et al. 2012).

6.2.5 Preference index calculation

Dietary preference was defined as the SSH decreasing rate at the beginning of grazing when herbage availability was ample, and constraints associated with diet choices were minimal (Hodgson 1979). As the dietary preference for a food choice can be affected by other choices that the animals have at the same time (Hodgson 1979), preference index is a value relative to the average of all the choices.

A growth model was used to fit the decline in perennial ryegrass SSH at different times during grazing, using SigmaPlot (version 13.0):

$$y = a(1 - e^{-bx}).$$

Consequently, the preference index, α was calculated as follow:

$$\alpha_i = \frac{y'_{(x=0)_i}}{\sum_j^8 y'_{(x=0)_j}/8}, \; i = 1, 2, \cdots 7, 8,$$

where y is the decline in SSH (cm), x is the time of grazing (h), a is the predicted final decline of SSH (cm), b is the fractional decreasing rate of sward height and $y'_{(x=0)}$ is the SSH decreasing rate at the beginning of grazing (x = 0).

At the beginning of grazing, among all the options the cows had, the faster the SSH decreased, the greater the preference index would be. A preference index of 1.00 would be representative of a cultivar if it is exactly intermediate, neither being preferred nor non-preferred among all the choices.

6.2.6 Selection index calculation

According to Manly (1974), when feeding by the consumer reduces the amount of food available, selection index, β could be calculated as follow:

$$\beta_i = ln(m_{posti}/m_{prei})/\sum_{j=1}^8 ln(m_{postj}/m_{prej}), \; j = 1, 2, \cdots 7, 8,$$

where m_{prei} and m_{posti} is the pre-grazing and post-grazing perennial ryegrass herbage mass of cultivar i, respectively.

A greater selection index means a cultivar was selected more by the consumer. A selection index of 0.125 (one out of the eight cultivar choices) would be representative of a cultivar if it is intermediate among all the choices.

6.2.7 Statistical analysis

The effects of white clover (main-factor), perennial ryegrass cultivar (sub-factor) and their interactions on sward structure, morphology characteristics, chemical composition, digestibility, preference index and selection index were analysed by ANOVA (SPSS version 22.0) for the each of the three phenological stages using a split-plot model:

$$y = \mu + \alpha_i + \gamma_k + \delta_{ik} + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijk},$$

where y is the dependent variable, μ is the overall mean, α_i is the effect of white clover, γ_k is the block effect, δ_{ik} is the interaction between white clover and block, β_j is the effect of perennial ryegrass cultivar, $(\alpha\beta)_{ij}$ is the interaction between white clover and perennial ryegrass cultivar and ε_{ijk} is the residual error.

White clover proportion and its petiole length in different perennial ryegrass cultivars were analysed in a randomised block design as they were applicable to mixtures only:

$$y = \mu + \beta_i + \gamma_k + \varepsilon_{ik},$$

where y is the dependent variable, μ is the overall mean, β_i is the effect of perennial ryegrass cultivar, γ_k is the block effect and ε_{ik} is the residual error.

Pearson correlation analysis was performed on SPSS (version 22.0) between the pasture measurements and dietary preference or selection index. Pasture measurements were converted to relative values according to the mean within a paddock to enable the comparison across paddocks and phenological stages.

6.3 Results

6.3.1 Sward structure and morphology

The inclusion of white clover had a negligible impact on the sward structure and morphology characteristics (Table 6.1, 6.2 and 6.3). Generally, the tetraploid cultivars Base and Bealey had lower herbage mass ($P < 0.05$) and heavier tillers ($P < 0.05$) with lower density ($P < 0.05$) than other cultivars. There was no effect of perennial ryegrass cultivar on the proportion of white clover at any of the three phenological stages ($P > 0.05$). There were no interactions ($P > 0.05$) between white clover and perennial ryegrass cultivar for sward structure and morphology.

6.3.2 Chemical composition and digestibility

When white clover was included in the sward, overall herbage CP concentration increased while the ADF and NDF concentrations decreased ($P < 0.05$, Table 6.1, 6.2 and 6.3). Tetraploid cultivars, Base and Bealey, had lower ADF and NDF concentrations than diploid cultivars at all three stages ($P < 0.05$). Generally, AberMagic had the highest WSC concentration. Alto, Commando and Kamo were low in DOMD.

6.3.3 Dietary preference and selection

Across all three experiments, the tetraploid cultivars, Base and Bealey, and the high-sugar diploid cultivar, AberMagic, had greater dietary preference and selection indices (preference index ranged from 1.02 to 1.45; selection index ranged from 0.13 to 0.18, Table 6.4) than the other cultivars. A significant interaction between perennial ryegrass cultivar and white clover for dietary preference index indicated that the dietary preference for perennial ryegrass cultivars was affected by the presence of white clover. With few exceptions, such as One50 at the pre-heading vegetative stage, dietary preference indices of almost all cultivars got closer towards the mean, 1.00, when white clover was present in the pasture at any of the three phenological stages. However, the ranking of perennial ryegrass cultivars remained similar regardless of the presence of white clover.

6.3.4 Correlations

The dietary preference index was negatively correlated with herbage mass, dead material proportion, tiller density, ADF and NDF concentration while positively correlated with SSH, the proportion of perennial ryegrass, lamina length, tiller mass of perennial ryegrass, OM and WSC concentration and DOMD ($P < 0.05$, Table 6.5). White clover and weed proportion, white clover petiole length and CP concentration were not correlated with preference ($P > 0.05$). Similar to the preference index, the selection index was correlated with perennial ryegrass proportion, tiller mass, tiller density and nutritive value, including ADF, NDF and WSC concentrations and DOMD ($P < 0.05$,

Table 6.5). However, the selection index was negatively correlated with the lower-layer sward structures, such as white clover petiole height ($P < 0.01$) and the proportion of dead material ($P < 0.01$), but not SSH ($P > 0.05$).

Table 6.1 Sward structure, morphology, chemical composition and digestibility of perennial ryegrass cultivars growing with and without white clover at the pre-heading vegetative stage.

	White clover (WC)			Perennial ryegrass cultivar (PRG)									*P* value		
	Absent	Present	SEM†	Base	Bealey	AberMagic	Alto	Commando	Kamo	One50	Prospect	SEM	WC	PRG	WC × PRG
Sward surface height (cm)	25.1	25.2	0.40	23.1^{d}	22.6^{d}	23.2^{d}	25.0^{c}	28.6^{a}	28.9^{a}	22.7^{d}	26.9^{b}	0.53	0.90	< 0.01	0.70
Herbage mass (kg DM/ha)	3460	3234	166	2690^{d}	2652^{d}	3194^{c}	3580^{bc}	4135^{a}	3958^{ab}	3320^{c}	3249^{c}	155	0.41	< 0.01	0.40
Perennial ryegrass (%)	81.0	75.4	0.64	78.4	79.2	79.4	77.6	78.8	76.1	79.3	76.8	1.00	0.01	0.18	0.27
White clover (%)	-	6.4	-	5.7	8.2	7.4	4.6	6.1	7.2	4.7	7.6	1.86	-	0.79	-
Weeds (%)	1.8	1.5	0.48	2.4^{ab}	2.9^{a}	1.9^{abc}	2.0^{abc}	0.9^{c}	1.1^{bc}	1.1^{bc}	1.3^{bc}	0.44	0.70	0.02	0.60
Dead material (%)	17.1	16.7	0.46	16.4^{bc}	13.8^{d}	15.0^{cd}	18.1^{ab}	17.3^{abc}	19.2^{a}	17.3^{abc}	18.0^{ab}	0.78	0.53	< 0.01	0.56
Tiller mass (mg)	56.2	46.0	1.97	55.9^{b}	67.5^{a}	44.3^{d}	55.7^{b}	47.5^{cd}	38.6^{e}	52.4^{bc}	47.0^{cd}	1.99	0.04	< 0.01	0.35
Tiller density (m^{-2})	5158	5668	154.7	3747^{e}	3025^{f}	5904^{c}	5143^{d}	6916^{b}	7997^{a}	5046^{d}	5526^{cd}	237.5	0.10	< 0.01	0.81
Ryegrass lamina length (cm)	13.5	13.1	0.10	14.2^{a}	13.8^{ab}	11.6^{c}	14.0^{ab}	13.7^{ab}	13.2^{ab}	12.9^{abc}	12.8^{bc}	0.42	0.06	< 0.01	0.61
White clover petiole length (cm)	-	11.1	-	9.1^{b}	9.5^{b}	11.7^{a}	11.9^{a}	12.6^{a}	12.7^{a}	10.0^{b}	11.7^{a}	0.60	-	< 0.01	-
OM (g/kg DM)	907	903	1.1	904^{bcd}	905^{bcd}	912^{a}	901^{cd}	908^{ab}	900^{d}	907^{abc}	903^{bcd}	2.1	0.07	0.00	0.44
CP (g/kg DM)	154	171	1.7	166	174	163	159	158	161	154	163	4.5	0.01	0.13	0.28
WSC (g/kg DM)	232	214	1.3	217^{bcd}	224^{bc}	252^{a}	211^{cd}	223^{bc}	196^{d}	241^{ab}	221^{bcd}	8.5	0.00	< 0.01	0.28
ADF (g/kg DM)	248	243	1.0	245^{c}	234^{d}	234^{d}	252^{b}	254^{b}	261^{a}	241^{c}	243^{c}	2.0	0.03	< 0.01	0.10
NDF (g/kg DM)	487	471	2.6	472^{c}	456^{d}	464^{cd}	490^{b}	499^{a}	507^{a}	468^{c}	473^{c}	3.5	0.02	< 0.01	0.22
DOMD (%)	73.5	73.5	0.23	73.9^{cd}	75.2^{ab}	75.8^{a}	72.5^{de}	72.0^{e}	70.3^{f}	74.6^{bc}	73.6^{cd}	0.41	0.94	< 0.01	0.32

†SEM, standard error of the mean

Means followed by different superscripts are significantly different by Duncan's multiple range test ($P < 0.05$)

Table 6.2 Sward structure, morphology, chemical composition and digestibility of perennial ryegrass cultivars growing with and without white clover at the reproductive stage.

	White clover (WC)			Perennial ryegrass cultivar (PRG)									P value		
	Absent	Present	SEM†	Base	Bealey	AberMagic	Alto	Commando	Kamo	One50	Prospect	SEM	WC	PRG	WC × PRG
Sward surface height (cm)	24.0	23.6	0.44	23.6^{b}	25.4^{a}	24.6^{ab}	24.6^{ab}	23.3^{b}	20.7^{c}	23.2^{b}	25.4^{a}	0.49	0.53	< 0.01	0.05
Herbage mass (kg DM/ha)	3413	3589	202.9	2948^{d}	2990^{d}	3551^{bc}	3912^{ab}	3701^{ab}	3192^{cd}	4039^{a}	3675^{ab}	143.0	0.58	< 0.01	0.72
Perennial ryegrass (%)	81.2	77.3	0.65	82.5	79.4	79.6	78.0	79.2	75.6	79.4	80.3	1.18	0.02	0.02	0.03
White clover (%)	-	6.8	-	4.2	10.7	10.1	4.8	4.2	9.5	3.7	7.1	1.99	-	0.08	-
Weeds (%)	1.7	1.4	0.22	1.3^{b}	2.8^{a}	1.1^{b}	2.0^{ab}	1.2^{b}	1.4^{b}	1.5^{b}	0.9^{b}	0.36	0.44	0.02	0.13
Dead material (%)	17.1	14.6	0.72	14.1^{cd}	12.5^{d}	14.2^{cd}	17.6^{ab}	17.5^{ab}	18.3^{a}	17.3^{ab}	15.2^{bc}	0.88	0.09	< 0.01	0.77
Tiller mass (mg)	56.7	55.8	1.49	66.4^{a}	74.0^{a}	53.1^{bc}	55.7^{bc}	47.3^{cd}	39.9^{d}	57.6^{b}	55.8^{bc}	2.81	0.68	< 0.01	0.55
Tiller density (m^{-2})	5090	5195	202.3	3580^{c}	3140^{c}	5404^{b}	5424^{b}	6366^{a}	5870^{ab}	5596^{ab}	5762^{ab}	269.0	0.74	< 0.01	0.67
Ryegrass lamina length (cm)	13.1	12.0	0.49	12.0^{abc}	13.3^{ab}	11.8^{bc}	13.6^{ab}	12.6^{ab}	10.6^{c}	12.5^{ab}	13.7^{a}	0.56	0.21	0.01	0.17
White clover petiole length (cm)	-	12.0	-	10.8^{c}	10.6^{bc}	12.5^{ab}	11.5^{abc}	12.1^{abc}	12.8^{a}	12.5^{ab}	12.9^{a}	0.56	-	0.04	-
OM (g/kg DM)	902	901	3.8	910^{a}	904^{ab}	910^{a}	900^{bc}	893^{c}	893^{c}	905^{ab}	902^{ab}	2.0	0.86	< 0.01	0.03
CP (g/kg DM)	149	168	5.2	143^{c}	168^{ab}	156^{bc}	152^{bc}	174^{a}	166^{ab}	144^{c}	163^{ab}	5.5	0.08	< 0.01	0.16
WSC (g/kg DM)	189	181	14.0	220^{a}	194^{abc}	219^{a}	184^{bc}	141^{d}	145^{d}	203^{ab}	174^{c}	9.1	0.70	< 0.01	0.01
ADF (g/kg DM)	278	267	2.1	270^{bcd}	260^{d}	262^{cd}	276^{ab}	282^{a}	283^{a}	273^{abc}	274^{ab}	3.8	0.04	< 0.01	0.03
NDF (g/kg DM)	539	515	5.1	522^{bcd}	506^{d}	509^{cd}	531^{ab}	547^{a}	541^{ab}	527^{abc}	529^{ab}	6.5	0.05	< 0.01	0.03
DOMD (%)	67.0	68.5	0.61	69.4^{ab}	69.6^{a}	70.6^{a}	67.4^{c}	65.2^{d}	65.3^{d}	67.6^{bc}	67.5^{c}	0.60	0.20	< 0.01	0.01

†SEM, standard error of the mean

Means followed by different superscripts are significantly different by Duncan's multiple range test ($P < 0.05$)

Table 6.3 Sward structure, morphology, chemical composition and digestibility of perennial ryegrass cultivars growing with and without white clover at the post-flowering vegetative stage.

	White clover (WC)			Perennial ryegrass cultivar (PRG)									*P* value		
	Absent	Present	SEM†	Base	Bealey	AberMagic	Alto	Commando	Kamo	One50	Prospect	SEM	WC	PRG	WC × PRG
Sward surface height (cm)	17.5	18.4	0.14	17.1^{bc}	18.6^{a}	18.7^{a}	18.1^{ab}	17.7^{ab}	16.2^{c}	18.6^{a}	18.7^{a}	0.42	0.02	< 0.01	0.12
Herbage mass (kg DM/ha)	1399	1370	94.7	1088^{b}	1335^{a}	1540^{a}	1308^{ab}	1495^{a}	1413^{a}	1476^{a}	1445^{a}	77.9	0.85	0.01	0.07
Perennial ryegrass (%)	72.8	71	0.91	75.0^{a}	74.8^{a}	71.7^{ab}	70.5^{b}	69.3^{b}	73.1^{ab}	70.4^{b}	69.8^{b}	1.23	0.25	< 0.01	0.49
White clover (%)	-	4.9	-	5.2	6.7	6.3	3.3	3.4	5.1	3.5	5.6	1.65	-	0.70	-
Weeds (%)	0.8	1	0.35	0.8	1.5	1.2	0.8	0.9	0.7	0.7	0.9	0.30	0.75	0.62	0.03
Dead material (%)	26.3	23.1	1.11	21.6^{c}	20.4^{c}	24.0^{abc}	27.0^{ab}	28.4^{a}	23.6^{bc}	26.9^{ab}	26.5^{ab}	1.39	0.14	< 0.01	0.82
Tiller mass (mg)	25.7	27.2	0.47	24.7^{b}	37.9^{a}	26.1^{b}	25.5^{b}	23.1^{bc}	20.1^{c}	26.7^{b}	27.0^{b}	1.37	0.13	< 0.01	0.25
Tiller density (m^{-2})	4135	3801	278.0	3380	2702	4421	3742	4604	5198	3903	3863	206.8	0.49	< 0.01	0.85
Ryegrass lamina length (cm)	14	15.1	0.31	14.7^{abc}	16.1^{a}	14.3^{bc}	14.4^{abc}	13.2^{cd}	12.3^{d}	16.0^{a}	15.5^{ab}	0.51	0.11	< 0.01	0.14
White clover petiole length (cm)	-	10.2	-	8.9^{bc}	8.1^{c}	10.9^{ab}	10.5^{ab}	10.4^{ab}	10.6^{ab}	10.6^{ab}	11.8^{a}	0.69	-	0.03	-
OM (g/kg DM)	864	868	1.6	868^{abc}	868^{ab}	870^{a}	863^{bc}	862^{c}	869^{a}	865^{abc}	862^{c}	1.9	0.23	0.01	0.55
CP (g/kg DM)	230	249	4.0	252^{a}	254^{a}	227^{c}	227^{c}	231^{c}	245^{ab}	234^{bc}	245^{ab}	4.0	0.05	< 0.01	0.49
WSC (g/kg DM)	60	63	2.0	59^{bc}	71^{b}	86^{a}	60^{bc}	47^{c}	58b^{c}	61^{bc}	48^{c}	4.5	0.46	< 0.01	0.57
ADF (g/kg DM)	281	268	2.6	273^{bc}	265^{d}	270^{cd}	282^{a}	281^{a}	272^{c}	278^{ab}	274^{bc}	1.9	0.04	< 0.01	0.22
NDF (g/kg DM)	521	493	4.6	503^{cd}	489^{e}	499^{de}	519^{ab}	522^{a}	505^{cd}	515^{abc}	509^{bcd}	4.2	0.02	< 0.01	0.64
DOMD (%)	65.5	67.4	0.58	67.4^{a}	68.4^{a}	67.9^{a}	65.1^{c}	64.5^{c}	66.9^{ab}	65.7^{bc}	65.5^{bc}	0.50	0.12	< 0.01	0.47

†SEM, standard error of the mean

Means followed by different superscripts are significantly different by Duncan's multiple range test ($P < 0.05$)

Table 6.4 Effect of white clover on the dietary preference and selection of dairy cows for perennial ryegrass cultivars at the pre-heading vegetative, reproductive and post-flowering vegetative stages.

	White clover (WC)	Perennial ryegrass cultivar (PRG)									*P* value		
		Base	Bealey	AberMagic	Alto	Commando	Kamo	One50	Prospect	SEM†	WC	PRG	PRG × WC
	Pre-heading vegetative stage												
Preference index	Absent	1.29	1.24	1.36	0.74	0.89	0.72	0.76	1.00				
	Present	1.05	1.04	1.31	1.00	1.03	1.09	0.63	0.85				
	Mean	1.17^{ab}	1.14^{b}	1.34^{a}	0.87^{c}	0.96^{c}	0.91^{c}	0.70^{d}	0.92^{c}	0.060	-	< 0.01	< 0.01
Selection index	Absent	0.15	0.14	0.15	0.10	0.15	0.12	0.10	0.10				
	Present	0.15	0.17	0.12	0.14	0.10	0.12	0.10	0.10				
	Mean	0.15^{a}	0.15^{a}	0.13^{ab}	0.12^{ab}	0.13^{ab}	0.12^{ab}	0.10^{ab}	0.10^{b}	0.013	-	0.03	0.30
	Reproductive stage												
Preference index	Absent	1.09	1.67	1.14	0.89	0.83	0.38	0.71	1.31				
	Present	1.06	1.23	1.25	0.85	0.95	0.72	0.93	1.01				
	Mean	1.07^{bcd}	1.45^{a}	1.19^{ab}	0.87^{d}	0.89^{cd}	0.55^{e}	0.82^{d}	1.16^{bc}	0.091	-	< 0.01	0.05
Selection index	Absent	0.16	0.19	0.15	0.11	0.09	0.05	0.14	0.12				
	Present	0.17	0.14	0.14	0.11	0.09	0.11	0.12	0.12				
	Mean	0.16^{ab}	0.17^{a}	0.15^{ab}	0.11^{bcd}	0.09^{cd}	0.08^{d}	0.13^{abc}	0.12^{abcd}	0.016	-	<0.01	0.56
	Post-flowering vegetative stage												
Preference index	Absent	1.14	1.34	1.09	1.05	0.95	0.61	0.64	1.18				
	Present	0.89	1.26	0.99	0.86	1.12	0.79	1.16	0.94				
	Mean	1.02^{b}	1.30^{a}	1.04^{ab}	0.95^{bc}	1.04^{ab}	0.70^{c}	0.90^{bc}	1.06^{ab}	0.087	-	< 0.01	0.04
Selection index	Absent	0.12	0.16	0.17	0.11	0.12	0.12	0.11	0.09				
	Present	0.15	0.21	0.12	0.10	0.08	0.11	0.13	0.09				
	Mean	0.14^{bc}	0.18^{a}	0.15^{ab}	0.11^{bc}	0.10^{bc}	0.12^{bc}	0.12^{bc}	0.09^{c}	0.015	-	<0.01	0.41

†SEM, standard error of the mean

Means followed by different superscripts are significantly different by Duncan's multiple range test ($P < 0.05$)

Table 6.5 Pearson correlation coefficients between dietary preference and selection of dairy cows for perennial ryegrass cultivars and sward structure, morphology, chemical composition and digestibility of perennial ryegrass pastures (n = 190).

	Preference index		Selection index	
	r	P value	r	P value
Sward surface height	0.385	< 0.01	0.060	0.41
Herbage mass	-0.179	0.01	0.108	0.14
Perennial ryegrass percentage	0.223	< 0.01	0.215	<0.01
White clover proportion†	0.039	0.70	0.003	0.98
Weeds percentage	-0.006	0.93	0.027	0.71
Dead material percentage	-0.301	< 0.01	-0.268	<0.01
Tiller mass	0.340	< 0.01	0.375	<0.01
Tiller density	-0.288	< 0.01	-0.164	0.02
Ryegrass lamina length	0.234	< 0.01	0.186	0.01
White clover petiole length†	-0.073	0.48	-0.372	<0.01
OM	0.198	0.01	0.225	<0.01
CP	0.071	0.33	-0.033	0.65
WSC	0.142	0.05	0.342	<0.01
ADF	-0.337	< 0.01	-0.265	<0.01
NDF	-0.273	< 0.01	-0.261	<0.01
DOMD	0.310	< 0.01	0.315	<0.01

†n = 95 for white clover measurements

6.4 Discussion

6.4.1 Sward structure, morphology, chemical composition and digestibility

There was a marked effect of perennial ryegrass cultivar on sward structure, morphology, chemical composition and digestibility. In keeping with previous studies (Roegiers et al. 1988; Smith et al. 2001), the tetraploid cultivars, Base and Bealey, had lower SSH and herbage mass, fewer and heavier tillers, and lower tiller density. Further, consistent with previous studies (Moorby et al. 2006; Wims et al. 2013; Wims et al. 2017), the tetraploid cultivars, Base and Bealey, and the high-sugar diploid cultivar, AberMagic, had a greater digestibility and lower ADF and NDF concentrations. This result probably reflects that tetraploid cultivars have a lower ratio of the cell wall to cell content (Stewart & Hayes 2011), and the greater WSC concentration in the high-sugar cultivar may dilute herbage fibre concentration (Delagarde et al. 2000).

Growing white clover in mixed pastures had a negligible impact on perennial ryegrass sward structure and morphology, although it did affect some aspects of the overall herbage chemical composition, such as the CP, ADF and NDF concentrations. The increase in herbage CP concentration and the decrease in herbage fibre concentration were most likely due to the greater CP concentration and lower ADF and NDF concentrations of white clover than perennial ryegrass (Evans et al. 1996). However, the overall size of the white clover effect on perennial ryegrass sward structure and morphology was smaller, compared with that associated with perennial ryegrass cultivar. Further, the tiller density of perennial ryegrass did not reduce by the introduction of white clover as expected according to the previous study (McDonagh et al. 2017). A possible reason for the limited impact could be the low white clover proportion in mixtures (< 7% DM), which was, in turn, caused by the high annual nitrogen (N) application rate (325 kg N/ha/year), suppressing the growth of white clover and decreasing its proportion (Labuschagne et al. 2006; Hennessy et al. 2012).

It was noteworthy that there were limited effects of perennial ryegrass cultivar on white clover proportion, with no significant differences in the proportion of white clover among perennial ryegrass cultivars at all three phenological stages. Frame and Boyd (1986) hypothesised that perennial ryegrass cultivars with an open structure and a lower tiller density, such as tetraploid cultivars, could support more white clover than those diploid cultivars with a dense sward. However, that was not confirmed in this study, perhaps due to the low white clover proportions in the pasture (< 7% DM). Similarly, several previous studies in New Zealand (Rossi et al. 2014; Wims et al. 2017) reported that cultivar ploidy had little effect on the white clover proportion in perennial ryegrass-white clover pastures.

6.4.2 Preference and selection

This study examined the effect of white clover on dietary preference and selection for perennial ryegrass cultivars. Dietary preference and selection were measured by the decline in SSH and disappearance of herbage mass, respectively, during a short-term grazing. A feature of the results was that the differences in dietary preference among perennial ryegrass cultivars were reduced when white clover was present. This effect was most noticeable at the reproductive stage in late spring when the digestibility of the pasture was the lowest (Lee et al. 2012). Given the low overall proportion of white clover in the pasture, the result of reduced preference is surprising. The reduced preference probably reflects the partial preference that has been previously shown for white clover (Rutter et al. 2004b, a; Francis et al. 2006), diminishing the effect of perennial ryegrass cultivar *per se*. This result is supported by the research showing that dietary preference between perennial ryegrass with a high and low herbage N concentration was reduced when white clover was offered as another choice (Cosgrove et al. 2002).

There was a consistent effect of perennial ryegrass cultivar on preference of dairy cows, such that tetraploid cultivars (Base and Bealey) and high-sugar diploid cultivar (AberMagic) were the most preferred in all three experiments. This finding agrees with previous studies (Roegiers et al. 1988; Smit et al. 2006). Previous studies suggested that taller swards are preferred by livestock (Illius et al. 1992). Although there was a positive correlation between SSH and preference across cultivars, the tetraploid cultivars were not always taller than the diploid counterparts; thus, the preference for taller sward is unlikely to explain the strong preference shown for the tetraploid cultivars. In agreement with previous studies (Mayland et al. 2000; Smit et al. 2006), there were positive correlations between dietary preference and digestibility and the WSC concentration, and negative correlations between dietary preference and the ADF and NDF concentrations. Tetraploid cultivars were characterised by a greater WSC concentration and digestibility, and lower ADF and NDF concentrations, compared with other cultivars and all these factors have contributed to a high dietary preference.

Generally, cultivars with a greater dietary preference index were also the cultivars selected most frequently, despite the fact that dietary preference and selection indices were based on different measurements and calculations. However, there were no interactions between perennial ryegrass cultivar and the presence of white clover for dietary selection. The dietary selection index was negatively correlated with the lower-layer sward structures, such as white clover petiole height and the proportion of dead material instead of upper-layer sward surface height. This may suggested that a lower white clover height would encourage dairy cows to graze deeper and a greater proportion of dead material may suppress the selection (Cosgrove & Edwards 2007). Similar to preference, selection index was correlated with herbage nutritive value, but with a higher positive correlation with herbage WSC concentration. As the WSC accumulate mainly in the sward lower-layers (Waite & Boyd 1953), herbage WSC concentration affects dietary selection more substantially than preference (Chiy & Phillips 1999; Smith et al. 2001).

6.5 Conclusion

The tetraploid perennial ryegrass cultivars, Base and Bealey, and high-sugar diploid cultivar, AberMagic, were most preferred and selected by dairy cows. Although white clover had limited effect on sward structure and morphology of perennial ryegrass pastures, its presence reduced the differences in dietary preference for perennial ryegrass cultivars. However, it did not lead to a significant re-ranking of cultivars in order of dietary preference index. The proportion of perennial ryegrass, herbage WSC concentration and DOMD were positively correlated with dietary preference and selection indices, while negative correlations were found with the proportion of dead material and herbage fibre concentration.

Chapter 7

Milk production, grazing behaviour and nitrogen-use efficiency of dairy cows grazing perennial ryegrass cultivars allocated in the morning or afternoon

7.1 Introduction

Pasture is a key component to sustaining dairy production in grazing systems. Perennial ryegrass (*Lolium perenne* L.) is one of the most widely used grass species, especially in temperate regions, due to its outstanding herbage nutritive value and dry matter (DM) yield. A range of commercial perennial ryegrass cultivars have been developed to improve herbage DM yield, nutritive value and persistence (Lee et al. 2012). However, less information is available on how cultivars affect feeding value and animal performance (Wims et al. 2013), partly due to the cost of comparison. In recent years, new cultivars with an elevated concentration of water-soluble carbohydrates (WSC) have been produced for a greater milk production and nitrogen-use efficiency (NUE) of dairy cows (Miller et al. 2001; Lee et al. 2012).

A factor that may affect animal performance is the timing of herbage allocation. Herbage DM percentage and the concentration of WSC increase during the day, because of the moisture loss and net photosynthesis accumulation (Orr et al. 2001a). Accordingly, other chemical components, such as crude protein (CP), neutral detergent fibre (NDF) and acid detergent fibre (ADF) are diluted (Delagarde et al. 2000). Improved herbage digestibility has also been recorded in the afternoon (Huntington & Burns 2007; Brito et al. 2008). On the other hand, ruminants show a regular grazing pattern with the longest and most intense grazing event taking place in the afternoon (including dusk) (Gregorini 2012). Therefore, matching this grazing pattern with herbage nutritive value diurnal fluctuations by allocating fresh pastures in the afternoon might enhance the performance of livestock (Orr et al. 2001a; Gregorini et al. 2006). It may also improve microbial synthesis efficiency and NUE of dairy cows (Vibart et al. 2012), as afternoon herbage contains more WSC and less CP, providing more rapidly fermentable carbohydrates (Hristov et al. 2005) and reducing ammonia in the rumen (Tamminga 1996).

The objectives of this study were (1) to quantify the effect of perennial ryegrass cultivar and timing of herbage allocation on herbage nutritive value, milk production and grazing behaviour of mid-lactation cows, and (2) to investigate the interaction between perennial ryegrass cultivar and timing of herbage allocation.

7.2 Materials and methods

7.2.1 Experimental site and design

The experiment was conducted in irrigated pastures at the Lincoln University Research Dairy Farm, Canterbury, New Zealand (43°38'S, 172°27'E, 12 m above sea level). It was a 2 × 2 randomised block design with four treatments (perennial ryegrass AberMagic AR1 or Prospect AR37 allocated in the morning or afternoon). Perennial ryegrass cultivars AberMagic and Prospect were sown in adjacent strips (from 30 to 50 m in width) at 20 kg/ha in monocultures in May 2015. Pastures were grazed rotationally from August 2015 to January 2016 before the experiment started. All experimental areas were irrigated by centre-pivot irrigation according to the schedule organized by the farm management team. The annual nitrogen (N) application rate was 250 kg N/ha.

These perennial ryegrass cultivars were chosen to match previous chapters (Chapter 3 and 6). AberMagic and Prospect were similar in morphology (Table 3.2, 6.1, 6.2 and 6.3) and sward structure (Table 6.1, 6.2 and 6.3). However, as AberMagic was bred for an elevated concentration of WSC, AberMagic and Prospect were different in herbage chemical composition and digestibility (Table 3.7, 5.1, 6.1, 6.2, 6.3; Figure 4.1, 4.2, 4.3 and 4.4), especially in the WSC concentration, which expresses a dramatic diurnal variation (Figure 4.1, Table A.3).

7.2.2 Animal management

Forty-eight Friesian × Jersey dairy cows were blocked into 12 groups with four cows each, according to milksolids yield (1.56 ± 0.01 kg/cow/day), age (6.67 ± 0.12 years), days in milk (174 ± 4.5 days) and liveweight (529.6 ± 6.1 kg). The 12 groups were assigned randomly to three replicates of four treatments for a 10-day grazing experiment (4-day transition period and 6-day experimental period). Morning and afternoon herbage allocation groups were offered their daily allowances of fresh pasture after morning milking (0830 h) and afternoon milking (1630 h), respectively. Daily herbage allowance was 30 kg DM/cow above ground level for each group. Cows had *ad libitum* access to water.

7.2.3 Pasture measurements

To estimate herbage mass, a minimum of 30 pre- and 30 post-grazing sward compressed height measurements were taken using a rising plate meter (RPM, Jenquip, Feilding, New Zealand) in each grazing area on a daily basis. The RPM measurements were calibrated by measuring the compressed height of herbage within a 0.2 m^2 quadrat and then harvesting this herbage to ground level. Herbage samples were oven-dried at 60°C for 48 h and weighed. Due to the difference between pre- and post-grazing sward structure, linear regression was separately performed between RPM (0.5 cm units) and pre- or post-grazing herbage mass (kg DM/ha) (Figure 7.1):

$$\text{Herbage mass}_{pre} = 99.8 \times RPM + 1139,\ \ n = 191,\ \ R^2 = 0.53, P < 0.01,$$

$$\text{Herbage mass}_{post} = 147.1 \times RPM + 331, n = 117,\ \ R^2 = 0.48, P < 0.01.$$

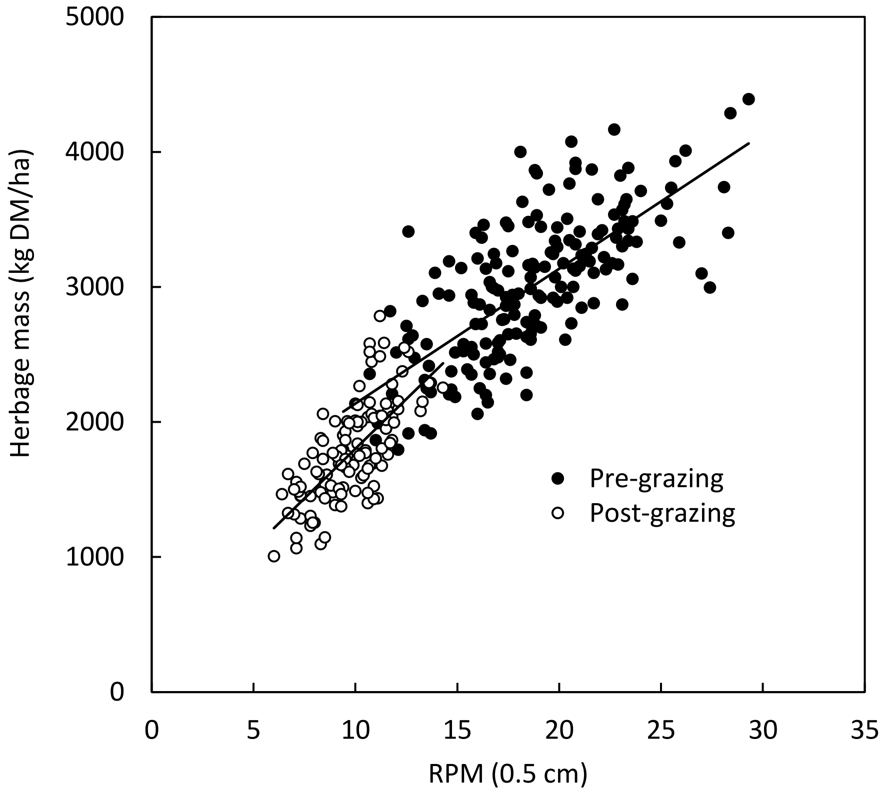


Figure 7.1 Calibrations between RPM measurements and herbage mass of pre-grazing and post-grazing swards.

Apparent herbage DM intake was calculated according to the difference between pre- and post-grazing herbage mass, the area allocated, and the number of cows grazed.

Herbage samples of approximately 500 g fresh weight were cut to ground level from at least 10 points in each group 1 h before herbage allocation. A subsample of about 50 g was weighed and oven-dried at 60°C for 48 h to obtain DM percentage. Another subsample of about 100 g was taken for botanical composition and morphological analysis. The subsample was sorted by hand into perennial ryegrass, dead material and weeds. Ten intact perennial ryegrass tillers were selected randomly, and the lengths of the newest fully expanded leaf blades (from ligule to tip) were recorded. All botanical fractions and tillers were oven-dried at 60°C for 48 h and weighed. Botanic composition and tiller mass were calculated on a DM basis. The remaining sample was frozen immediately, freeze-dried and ground through a 1-mm sieve for the evaluation of nutritive value by near infrared reflectance spectroscopy (NIRS Systems 5000, Foss, Maryland, USA) to determine the concentrations of organic matter (OM), CP, NDF and WSC and OM digestibility in dry matter (DOMD). NIRS calibrations were previously derived on perennial ryegrass samples (Jones & Hayward 1975;

MAFF 1986; AOAC 1990; van Soest et al. 1991). Assays for NDF excluded sodium sulphite or α-amylase and both ADF and NDF predictions were inclusive of residual ash (Bryant et al. 2012).

7.2.4 Grazing behaviour observation

Grazing behaviour of all cows was recorded by visual scan observation at 10-minute intervals over 3 h after morning and afternoon herbage allocation on day 7 and day 8. The behaviour was recorded when they were grazing (actively prehending herbage with the head lowered). Cumulative time spent on grazing was calculated, assuming activity recorded occurred over the 10 minutes period. Meanwhile, time spent on ten bites were recorded for two cows per group to calculate bite rate (bite per min). The average within each group was used for statistical analysis.

7.2.5 Milk measurements

Milk yield was measured daily for individual cows with an automated milking system (Alpro Herd management system, Tumba Sweden). Milk samples were collected daily from all cows at morning and afternoon milkings during the experimental period to determine milk composition and milk urea nitrogen (MUN). The samples for milk composition were analysed by the laboratory of Livestock Improvement Corporation Ltd. (Christchurch, New Zealand) to determine milk fat, protein and lactose by MilkoScan (Foss Electric, Hillerod Denmark). Samples for MUN measurement were centrifuged at 4,000*g* for 10 min to allow milk fats to solidify on the top and be removed. The skim milk was pipetted into a clean microcentrifuge tube and frozen at -20℃ before analysis. Skim milk was analysed by Daytona analyser (Randox Laboratories, Crumlin UK).

7.2.6 Statistical analysis

Data were averaged for each group across sampling dates. All analyses were performed using SPSS (version 22.0). Sward structure and morphological characteristics were analysed by one-way ANOVA using the following model:

$$y = \mu + \alpha_i + \gamma_j + \varepsilon_{ij},$$

where y is the dependent variable, μ is the overall mean, α_i is the effect of perennial ryegrass cultivar, γ_j is the block effect and ε_{ij} is the residual error.

Herbage chemical composition, digestibility, DM intake, milk yield and milk composition were analysed by two-way ANOVA using the following model:

$$y = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \gamma_k + \varepsilon_{ijk},$$

where y is the dependent variable, μ is the overall mean, α_i is the effect of perennial ryegrass cultivar, β_j is the effect of timing of herbage allocation, $(\alpha\beta)_{ij}$ is the interaction, γ_k is the block effect and ε_{ijk} is the residual error.

Grazing behaviour was analysed by three-way ANOVA using the following model:

$$y = \mu + \alpha_i + \beta_j + \epsilon_l + (\alpha\beta)_{ij} + (\beta\epsilon)_{jl} + (\alpha\epsilon)_{jl} + (\alpha\beta\epsilon)_{ijl} + \gamma_k + \varepsilon_{ijkl},$$

where y is the dependent variable, μ is the overall mean, α_i is the effect of perennial ryegrass cultivar, β_j is the effect of timing of herbage allocation, ϵ_l is the effect of time of grazing, and $(\alpha\beta)_{ij}$, $(\beta\epsilon)_{jl}$, $(\alpha\epsilon)_{jl}$ and $(\alpha\beta\epsilon)_{ijl}$ are the interactions, γ_k is the block effect and ε_{ijk} is the residual error.

7.3 Results

There were no significant differences in herbage mass and botanical composition between cultivars (*P* < 0.05, Table 7.1). However, AberMagic tiller mass was heavier than Prospect (48.4 vs. 43.1 mg DM, *P* < 0.05). Herbage DM percentage (22.3% vs. 18.8%, *P* < 0.01), WSC concentration (179 and 154 g/kg DM, *P* < 0.05) and DOMD (73.5% vs. 72.1%, *P* < 0.01) were all greater in the afternoon (Table 7.2). Although the WSC concentration was greater in AberMagic than Prospect (*P* < 0.05), perennial ryegrass cultivar has a tendency to interact with timing of herbage allocation (*P* = 0.07, SEM = 9.1). The concentration of WSC increased more during the day in Prospect than AberMagic (from 131 to 176 g/kg DM in Prospect; from 178 to 183 g/kg DM in AberMagic). Herbage DM percentage did not differ between cultivars (*P* > 0.05). Both cultivar and timing of herbage allocation did not significantly change herbage OM percentage, CP and NDF concentrations (*P* > 0.05, Table 7.2).

Apparent herbage DM intake, milk yield, milksolids yield and milk composition were unaffected (*P* > 0.05) by perennial ryegrass cultivar or timing of herbage allocation (Table 7.3). A significant interaction effect on MUN concentration (*P* = 0.05, SEM = 0.21) indicated that the effect of herbage allocation timing on MUN concentration was different between perennial ryegrass cultivars. MUN concentration decreased from 9.33 to 8.39 mmol/L when Prospect was allocated in the afternoon, while it was similar whether AberMagic was offered in the morning or afternoon (8.95 vs. 8.96 mmol/L).

There was no effect of cultivar or the timing of herbage allocation or the time of grazing on bite rate (mean = 57.9 min^{-1}, *P* < 0.05, Table 7.4). Cows grazing on Prospect tended to have a longer grazing time during 6-h grazing than cows grazing on AberMagic (161 vs. 147 min, *P* = 0.08). The interaction between timing of herbage allocation and time of grazing for grazing time tended to be significant (*P* = 0.06), suggesting that dairy cows tended to spend longer time grazing when they had the fresh herbage allocations regardless of morning or afternoon.

Table 7.1 Sward structure and morphological characteristics of perennial ryegrass cultivars AberMagic and Prospect.

	AberMagic	Prospect	SEM†	*P* value
Pre-grazing herbage mass (kg DM/ha)	2816	2708	22.6	0.08
Perennial ryegrass (%)	87.2	86.0	0.78	0.40
Weeds (%)	2.5	2.3	0.29	0.71
Dead material (%)	10.3	11.7	0.56	0.23
Post-grazing herbage mass (kg DM/ha)	1711	1718	48.6	0.92
Tiller density (m^{-2})	5197	5336	41.0	0.14
Tiller weight (mg DM)	48.4	43.1	0.87	0.05
Leaf length (cm)	18.0	19.0	0.27	0.12

†SEM, standard error of the mean

Table 7.2 DM percentage (%), chemical composition (g/kg DM) and digestibility (%) of perennial ryegrass cultivars allocated for grazing in the morning or afternoon.

	Cultivar (C)		Timing of herbage allocation (T)		SEM†	*P* value		
	AberMagic	Prospect	Morning	Afternoon		C	T	C × T
DM	21.0	20.1	18.8	22.3	0.41	0.16	< 0.01	0.62
OM	801	843	821	823	20.8	0.21	0.97	0.18
CP	152	157	158	151	6.3	0.57	0.47	0.16
WSC	180	153	154	179	6.4	0.02	0.04	0.07
NDF	444	476	466	453	14.1	0.16	0.54	0.18
DOMD	74.2	71.4	72.1	73.5	0.26	< 0.01	0.01	0.09

†SEM, standard error of the mean

Table 7.3 DM intake (kg/cow/day), milk yield (kg/cow/day), milksolids yield (kg/cow/day), milk composition (%) and MUN concentration (mmol/L) of dairy cows grazing perennial ryegrass cultivars allocated in the morning or afternoon.

	Cultivar (C)		Timing of herbage allocation (T)		SEM†	*P* value		
	AberMagic	Prospect	Morning	Afternoon		C	T	C × T
DM Intake	12.1	12.0	12.4	11.7	0.32	0.87	0.17	0.31
Milk yield	17.2	17.1	17.0	17.3	0.31	0.90	0.41	0.36
Fat	5.38	5.44	5.39	5.42	0.134	0.77	0.89	0.91
Protein	4.08	3.94	3.97	4.06	0.053	0.10	0.25	0.54
Lactose	4.95	5.02	5.00	4.96	0.032	0.19	0.44	0.45
Milksolids yield	1.61	1.59	1.57	1.64	0.026	0.57	0.10	0.41
MUN	8.96	8.87	9.13	8.69	0.151	0.45	0.12	0.05

†SEM, standard error of the mean

Table 7.4 Morning and afternoon bite rate (min^{-1}) and grazing time (min) of dairy cows grazing perennial ryegrass cultivars allocated in the morning or afternoon.

Cultivar (C)	Timing of herbage allocation (T)	Time of grazing (TG)	Bite rate†	Grazing time†
AberMagic	Morning	Morning	58.0	152
		Afternoon	60.7	152
	Afternoon	Morning	57.8	135
		Afternoon	58.8	148
Prospect	Morning	Morning	53.9	175
		Afternoon	58.3	148
	Afternoon	Morning	57.1	153
		Afternoon	58.6	168
SEM‡			3.56	13.9
P value				
C			0.20	0.08
T			0.91	0.35
TG			0.29	1.00
C × T			0.29	0.42
C × TG			0.59	0.42
T × TG			0.70	0.06
C × T × TG			0.67	0.35

†Bite rate and grazing time were observed for three hours
‡SEM, standard error of the mean

7.4 Discussion

7.4.1 Sward structure and nutritive value

Few studies comparing dairy cow performance on different perennial ryegrass cultivars have been able to separate the effect of morphological attributes from herbage chemical composition. In the current study, the two cultivars, AberMagic and Prospect, were similar in morphology (Table 3.2, 6.1, 6.2 and 6.3) and sward structure (Table 6.1, 6.2 and 6.3). Similar physical attributes for AberMagic and Prospect were also observed by Rossi et al. (2014), who compared eight perennial ryegrass cultivars, including AberMagic and Prospect, and classified them as the same functional group characterised by a high tiller density and fine leaf material. In terms of chemical composition, as a high-sugar cultivar, AberMagic, had a greater WSC concentration than Prospect, which is consistent with the results of previous studies (Staerfl et al. 2012; Cosgrove et al. 2014).

There was a pronounced effect of time of day on herbage chemical composition and digestibility. In keeping with previous research (Delagarde et al. 2000; Gregorini et al. 2006; Pulido et al. 2015), the DM percentage and WSC concentration were greater in the afternoon than in the morning, due to water loss via transpiration and carbohydrate accumulation via photosynthesis. Also, in agreement with previous studies (Huntington & Burns 2007; Brito et al. 2008), the enhanced WSC concentration improved herbage digestibility in the afternoon. Delagarde et al. (2000) noted that the amounts of CP and NDF (absolute mass) were similar in the morning and evening and their concentrations were thus passively diluted by the increasing amount of WSC during the day. However, in the present study, the increment in the WSC concentration might not have been great enough to significantly dilute the concentrations of CP and NDF within the herbage DM.

There was tentative evidence of an interaction between perennial ryegrass cultivar and time of day for the WSC concentration, with a greater increase in the WSC concentration in Prospect during the day than in AberMagic. However, the greater initial WSC concentration in AberMagic in the morning led to a greater overall WSC concentration in AberMagic than Prospect. It is unknown why a greater increase in the WSC concentration occurred in Prospect. However, it is possible that this is caused by the greater net photosynthesis rate in Prospect and the feedback inhibition of photosynthesis due to WSC accumulation in AberMagic (Rogers et al. 1998).

7.4.2 Intake and milk production

There were negligible effects of perennial ryegrass cultivar on milk production or composition. Wims et al. (2013) noted that cows grazing on AberMagic had lower milk yield and milksolids yield than cows grazing on Bealey, the tetraploid cultivar. Compared with normal-sugar diploid cultivars, dairy cows grazing the high-sugar cultivar, AberMagic, produced more milk (Miller et al. 2001). However, Cosgrove et al. (2007) and Moorby et al. (2006) found little differences in milk production between cows grazing the high-sugar and normal-sugar perennial ryegrass cultivars. The lack of effect on milk production observed in current study reflects that the differences in DM intake between cultivars were not significant (12.1 vs. 12.0 kg DM/cow/day) or that the differences in herbage WSC concentration and DOMD were not large enough (180 vs. 153 g/kg DM; 74.2% vs. 71.4%) to alter animal performance (Francis et al. 2006; Edwards et al. 2007).

Offering a new herbage allocation in the afternoon was expected to lead to a greater DM intake because the longest and most intense grazing events occur in the afternoon and near dusk (Orr et al. 1997; Gregorini 2012). However, in this study, DM intake and milksolids yield were found to be similar for the morning and afternoon herbage allocation systems. These findings were supported by previous studies reporting that the similar DM intake in the morning and afternoon herbage allocation system has led to the similar milk production (Abrahamse et al. 2009). The failure to detect any differences in DM intake and milk production may reflect the low overall herbage allowance which may have limited prospects for cows to increase their DM intake.

In the present study, bite rates and grazing time were similar in all treatments. However, dairy cows tended to spend more time grazing when they received a fresh pasture allowance regardless of morning or afternoon. Similarly, previous research showed that the proportion of time spent grazing was greater following the allocation of a fresh pasture strip (Abrahamse et al. 2009; Pulido et al. 2015). Pulido et al. (2015) also found that cows spent a similar amount of time grazing in the morning and afternoon if fresh herbage were allocated both in the morning and afternoon.

7.4.3 Milk urea nitrogen

MUN was measured in this study as an indicator of NUE (Kauffman & St-Pierre 2001). There was an interaction between cultivar and timing of herbage allocation for MUN concentration. MUN concentration was lower when Prospect was allocated in the afternoon than the morning, indicating a better NUE, while MUN concentration remained similar regardless of when AberMagic was allocated. Edwards et al. (2007) noted that herbage WSC:CP is related to NUE of dairy cows, which could be the explanation of this interaction. In this study, there was a greater increase in the WSC concentration from morning to afternoon in Prospect than in AberMagic, while the CP concentration did not change significantly between cultivars or timing of herbage allocation. As a result, Prospect's WSC:CP ratio increased from 0.78 in the morning to 1.20 in the afternoon, while AberMgic's WSC:CP ratio was 1.20 in the morning and 1.17 in the afternoon. On the other hand, compared with Prospect, AberMagic did not lead to a lower MUN concentration even with a greater overall herbage WSC:CP ratio. This indicates other factors such as N fractionation and digestibility, could also contribute to NUE, in addition to WSC:CP ratio (Cosgrove et al. 2007; Edwards et al. 2007). Therefore, cultivars with a greater WSC concentration diurnal variation might be more suitable for the afternoon herbage allocation system to improve NUE of dairy cows.

7.5 Conclusion

AberMagic and herbage allocated in the afternoon had a greater WSC concentration and digestibility. However, there was little effect of perennial ryegrass cultivar and herbage allocation timing on milk production of mid-lactation dairy cows, due to no significant changes in DM intake. Dairy cows tended to spend more time grazing when they received fresh pasture allowance regardless in the morning or afternoon. The interaction observed for MUN concentration between cultivar and timing of herbage allocation indicates that the choice of cultivar and herbage allocation timing could potentially improve NUE of dairy cows. Cultivar × management interactions should be considered in both cultivar evaluation and management strategy.

Chapter 8

General discussion

This thesis evaluated perennial ryegrass (*Lolium perenne* L.) cultivars under different pasture management (such as growing in monocultures or mixtures with white clover (*Trifolium repens* L.) and defoliating at different stages of regrowth and times of day). The evaluation of herbage dry matter (DM) yield and the interaction between perennial ryegrass cultivar and pasture management were addressed by Rossi (2016). The present thesis has focused on the evaluation based on feeding value related factors for dairy production, including morphology (Chapter 3), herbage nutritive value (Chapter 3 & 4), rumen degradability (Chapter 5), dietary preference (Chapter 6) and milk production (Chapter 7). By summarising the outcome of previous experiment chapters, this general discussion aims to compare the effect of cultivar and pasture management on perennial ryegrass, to investigate cultivar × management interactions, to explore the implications and to propose areas of future work.

8.1 Cultivar and management effects

Cultivar and management effects on perennial ryegrass morphology, sward structure, herbage chemical composition, digestibility, rumen degradation, dietary preference and selection, milk production and nitrogen-use efficiency (NUE) of dairy cows across Chapter 3 to 7 are summarised in Figure 8.1, 8.2, 8.3, 8.4, 8.5 and 8.7. In order to make data comparable across chapters and to provide indications of relative effects, original data were normalised against their overall average. Original values can be found in the corresponding chapters. Dietary preference and selection indices were not normalised because they are relative values. Since water-soluble carbohydrates (WSC):crude protein (CP) and milk urea nitrogen (MUN) concentration were used as predictors of NUE, their absolute values were shown without normalisation.

8.1.1 Morphology and sward structure

According to Chapter 3 and 6, perennial ryegrass cultivars were significantly different in morphology and sward structure. Tetraploid cultivars always had a greater lamina proportion and a lower tiller density (Figure 8.1). In line with previous results (Phillips & James 1998; Ribeiro Filho et al. 2005), the presence of white clover had less influence on perennial ryegrass lamina proportion, leaf length, sward surface height and tiller density than perennial ryegrass cultivar (Figure 8.1). This may reflect low proportions of white clover in the swards (< 7% DM above ground level) and thus the little opportunity to affect perennial ryegrass morphology. Also, Yamada et al. (2004) reported that quantitative trait loci are responsible for morphological traits in perennial ryegrass, including plant height, tiller size, leaf length and leaf width, with little environmental impact. In summary, perennial ryegrass morphology and sward structure were largely depending on perennial ryegrass cultivar, rather than the presence of white clover. There was a greater effect of regrowth stage than perennial ryegrass cultivar on white clover proportion.

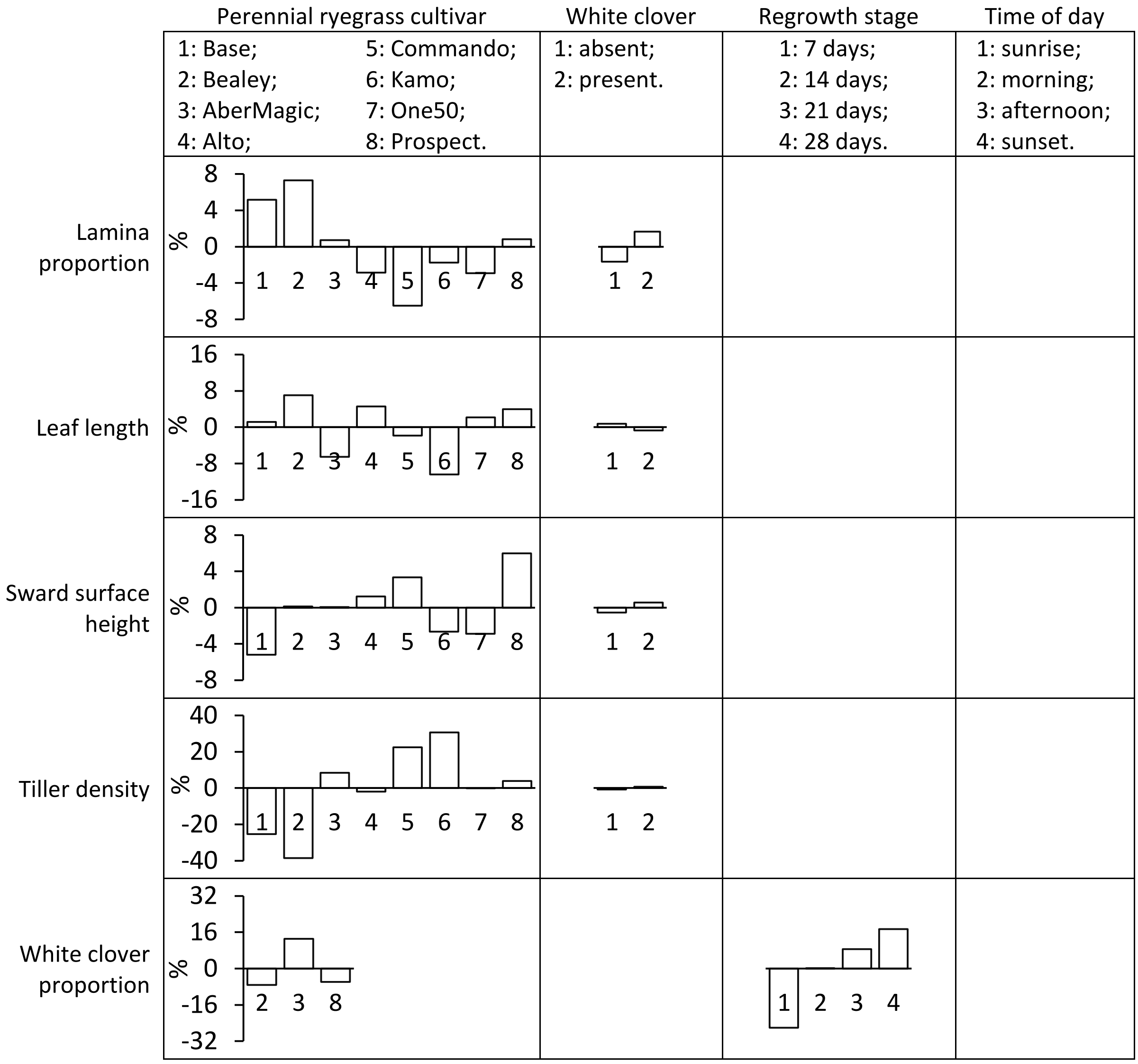


Figure 8.1 Summary of the effect of cultivar and management on morphology and sward structure of perennial ryegrass pastures (values were normalised against the average).

8.1.2 Chemical composition and digestibility

In this study, perennial ryegrass cultivars differed significantly from each other in chemical composition and digestibility (Figure 8.2). The high-sugar cultivar, AberMagic, had the greatest concentration of WSC in all experiments. Other chemical components, such as CP and NDF are diluted accordingly (Delagarde et al. 2000). In line with previous studies (Moorby et al. 2006; Wims et al. 2013; Wims et al. 2017), the tetraploid cultivars always had lower ADF and NDF concentrations and a greater digestibility compared with diploid cultivars, due to their lower ratio of the cell wall to cell content (Stewart & Hayes 2011).

The presence of white clover exerted minor effects on herbage DOMD (Figure 8.2), but it increased CP and reduced the NDF concentration of perennial ryegrass pastures to a similar extent as perennial ryegrass cultivar (Figure 8.2). However, the regrowth stage and time of day played a more substantial role than both perennial ryegrass cultivar and the presence of white clover on herbage nutritive value (Figure 8.2). From day 7 to day 28 of pasture regrowth, on average, herbage WSC and NDF concentration increased by 98 and 43 g/kg DM, respectively, but the CP concentration and DOMD decreased by 50 g/kg DM and 6%, respectively. During the day (from sunrise to sunset), on average, the WSC concentration and DOMD increased by 87 g/kg DM and 5%, while the CP and NDF concentration decreased by 30 and 61 g/kg DM, respectively.

Previous studies found differences in herbage nutritive value among perennial ryegrass cultivars (Smith et al. 2001; McGrath et al. 2014; Beecher et al. 2015; Ganche et al. 2015). Some of those results, at least in part, were due to the effect of defoliation timing rather than cultivar itself. For example, Moorby et al. (2006) harvested normal-sugar perennial ryegrass cultivar at 1000 h and the high-sugar cultivar at 1400 h to investigate the effect of the high-sugar perennial ryegrass cultivar on dairy production. The purpose of delayed harvesting of high-sugar cultivar was to emphasis on the great WSC concentration (Edwards et al. 2007). However, the effect of time of day would confound the effect of cultivar, potentially leading to an incorrect conclusion on the expression of high-sugar trait. In summary, pasture management sometimes was more effective in manipulating herbage chemical composition and digestibility than perennial ryegrass cultivars. Consequently, the evaluation of the nutritive value of perennial ryegrass cultivars need to be conducted in a defined and consistent situation to exclude the effect of pasture management.

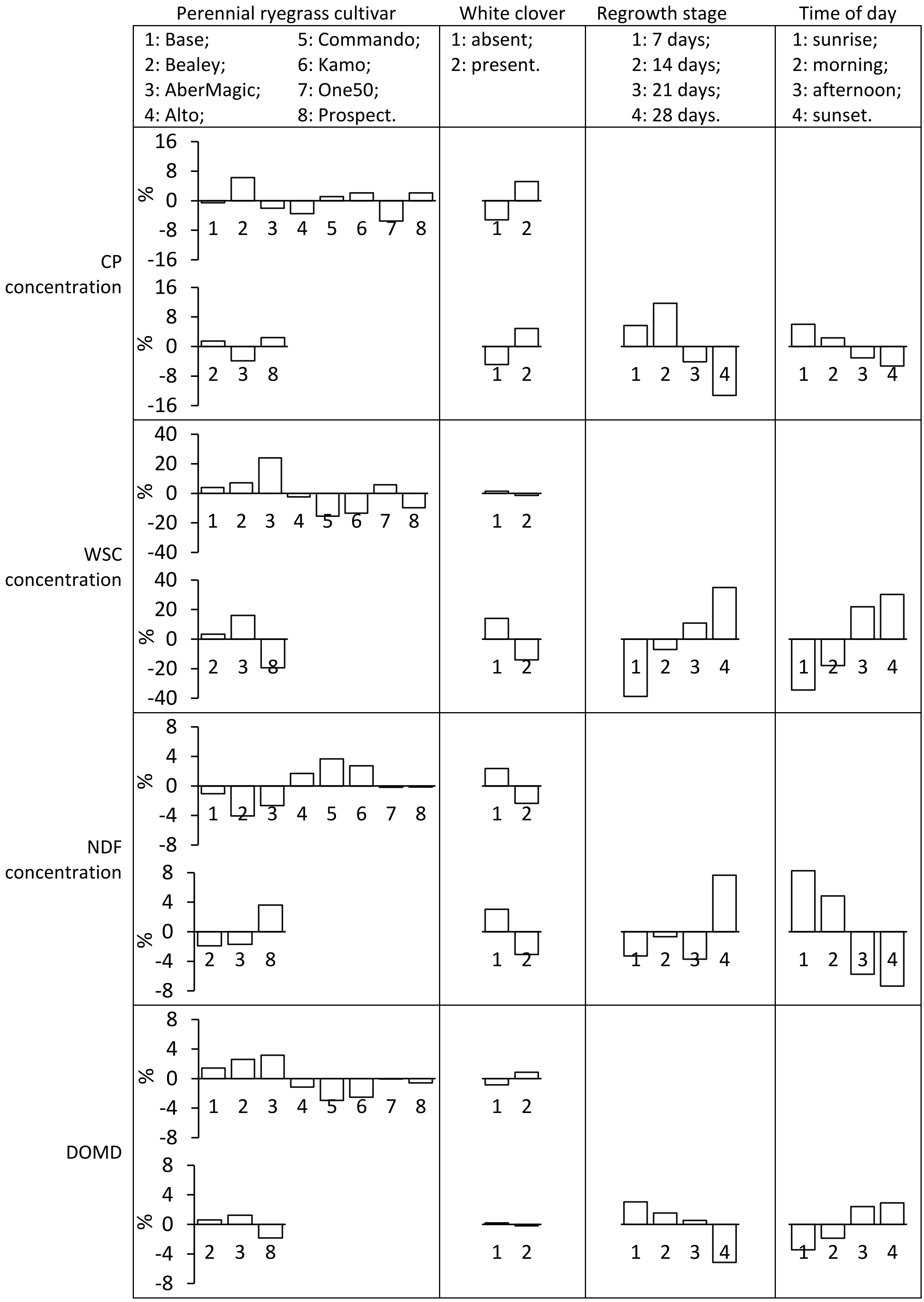


Figure 8.2 Summary of the effect of cultivar and management on chemical composition and digestibility of perennial ryegrass pastures (values were normalised against the average).

8.1.3 Rumen degradation

Perennial ryegrass cultivar exerted a greater effect on organic matter (OM) rumen degradation than the presence of white clover (Figure 8.3). The tetraploid cultivar, Bealey, had a greater soluble fraction, *a*, and faster insoluble-degradable fraction, *b*, of OM, which were considered as a selection criterion in forage plant breeding (Sun & Waghorn 2012). Therefore, the effective degradability of OM was greatest in Bealey, followed by the high-sugar diploid cultivar, AberMagic, and the normal-sugar diploid cultivar, Prospect. On the other hand, the presence of white clover had a more pronounced effect on effective rumen degradable protein than perennial ryegrass cultivar (Figure 8.3). This was due to the combined effect of white clover's greater effective degradability of OM and CP, and its outstanding CP concentration.

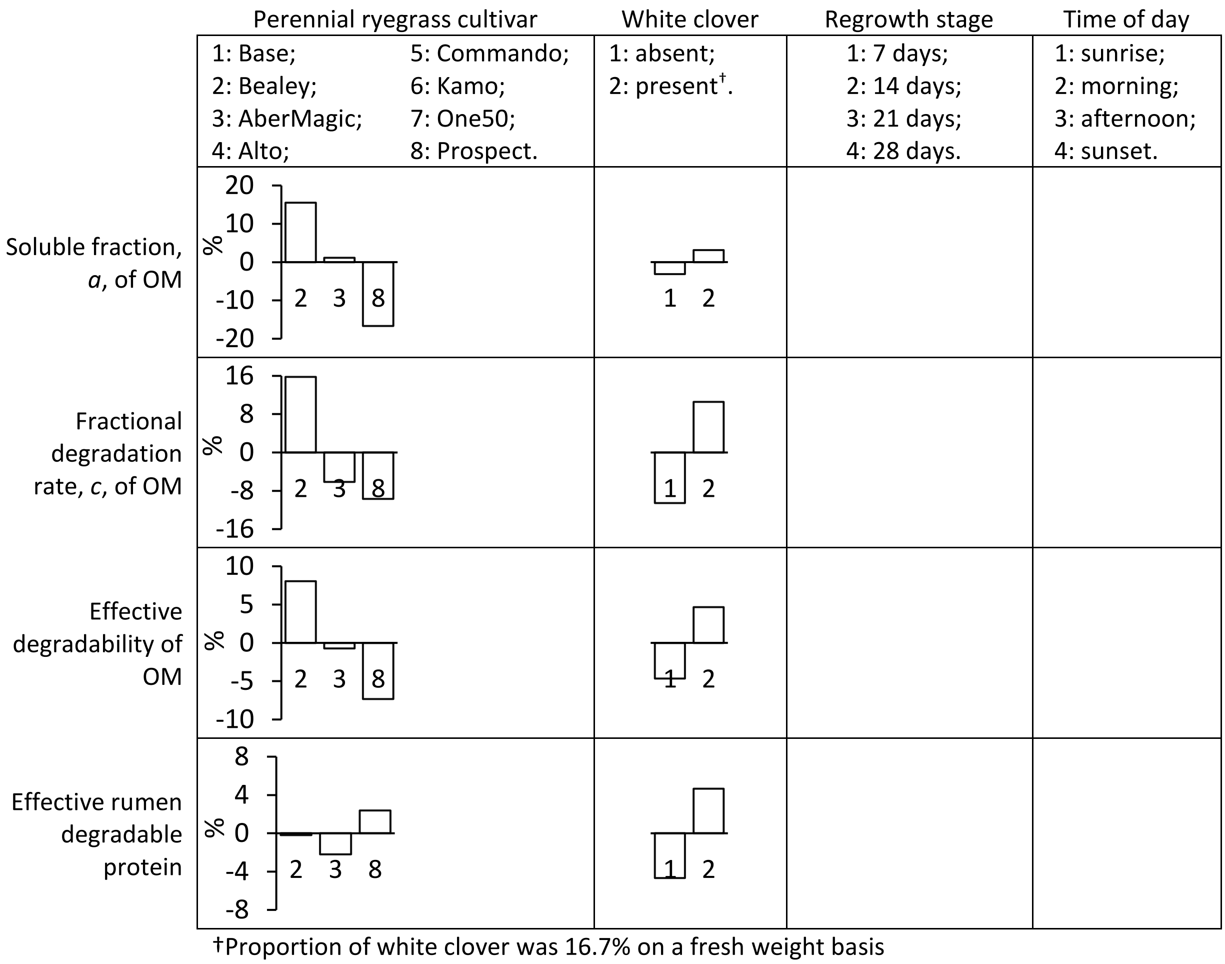


Figure 8.3 Summary of the effect of cultivar and management on rumen degradation characteristics of perennial ryegrass pastures (values were normalised against the average).

8.1.4 Dietary preference and selection

In this study, dairy cows expressed consistent dietary preference and selection for perennial ryegrass cultivars. The tetraploid perennial ryegrass cultivars, Base and Bealey, and high-sugar diploid cultivar, AberMagic, were the most preferred and selected cultivars (Figure 8.4). The differences in dietary preference and selection between perennial ryegrass monocultures and perennial ryegrass-white clover mixtures were not compared in this study. However, dairy cows show a partial preference for white clover over perennial ryegrass (Rutter et al. 2004b, a; Francis et al. 2006), which potentially makes white clover exert significant effects on dietary preference for perennial ryegrass cultivars (see 8.2.1).

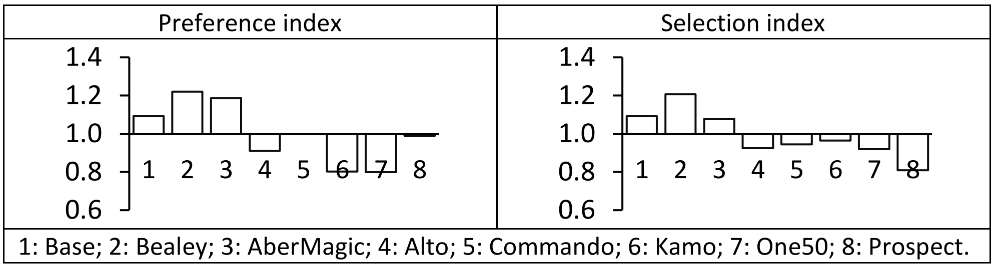


Figure 8.4 Summary of the effect of cultivar on dietary preference and selection.

8.1.5 Milk production

Compared with other diploid cultivars, the high-sugar cultivar, AberMagic, had greater lamina proportion, herbage WSC concentration and DOMD, and was highly preferred (Chapter 3, 4 and 6). However, these did not lead to a significant increase in milk production of mid-lactating dairy cows (Chapter 7). The lack of effect of perennial ryegrass cultivar on milk production reflects that the differences in DM intake between cultivars were not significant due to the low herbage allocation. However, in the afternoon herbage allocation system, the tendency of lower DM intake and greater milksolids yield suggested a greater feed conversion efficiency due to the greater herbage DOMD in the afternoon.

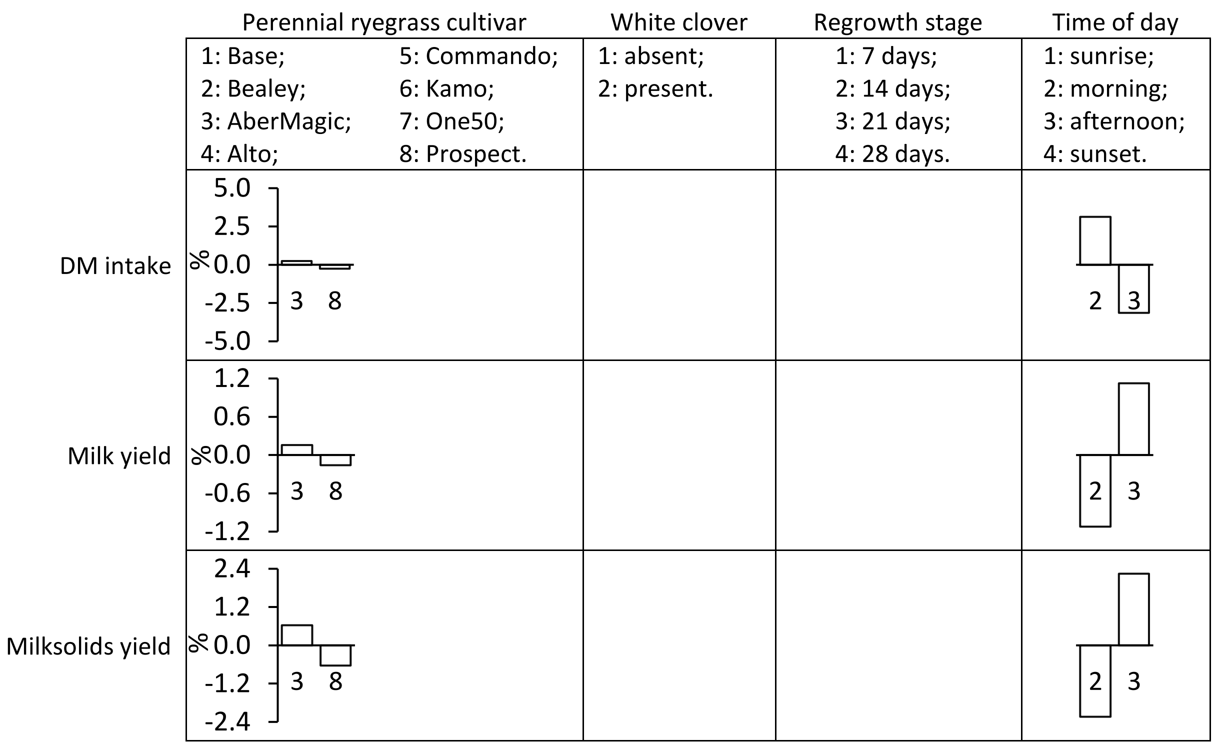


Figure 8.5 Summary of the effect of cultivar and management on DM intake, milk yield and milksolids yield (values were normalised against the average).

8.1.6 Nitrogen-use efficiency

In this study, herbage WSC:CP and MUN concentration were used to indicate NUE of dairy cows. Rapidly fermentable carbohydrates (such as WSC) are critical as an energy source for microorganisms in the rumen to capture N as microbial protein and reduce the N loss in the urine (Miller et al. 2001). Edwards et al. (2007) proposed WSC to CP ratio (WSC:CP) as an index to predict NUE for dairy cows in pasture-based systems. Urea excretion in urine is directly proportional to the concentration of urea in blood, which is further proportional to the concentration of MUN. Therefore, MUN concentration could also be a good predictor of NUE (Kauffman & St-Pierre 2001). Previously, the relationships of WSC:CP and MUN against NUE of dairy cows were established for New Zealand pasture-based grazing system (Figure 8.6). The fomulas in Figures 8.6 were used to predict NUE in Figure 8.8.

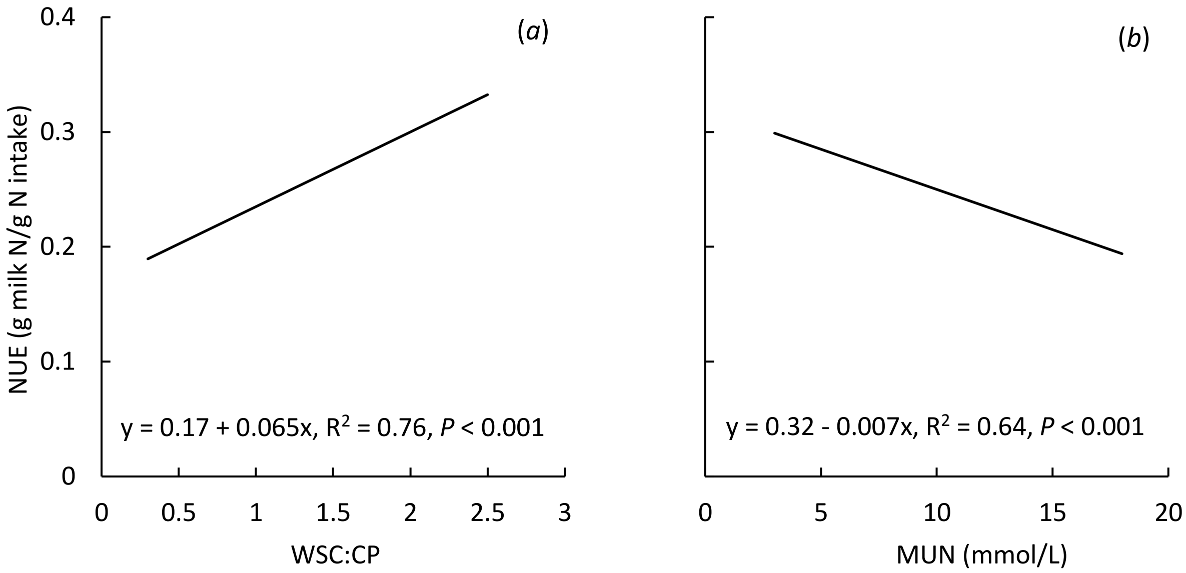


Figure 8.6 Relationships of herbage WSC:CP (*a*) and MUN (*b*) against NUE of dairy cows (Aizimu et al. 2013).

AberMagic is a high-sugar cultivar that was bred for an elevated concentration of WSC and expressed a greater WSC concentration (Chapter 3, 4, 5, 6 and 7). Additionally, a greater WSC concentration was accompanied by a lower CP concentration (Chapter 3, 4, 5, 6 and 7). Therefore, WSC:CP was greater in AberMagic than all the other cultivars (Figure 8.7). Although the presence of white clover had less of an effect on herbage WSC:CP than perennial ryegrass cultivar, it could decrease herbage WSC:CP (Figure 8.7 and 8.8). This is because white clover was characterised by a greater CP concentration and a lower concentration of WSC than perennial ryegrass (Chapter 3) and it increased the CP concentration of accompanying perennial ryegrass (Chapter 3). As reported in Chapter 3, perennial ryegrass lamina had twice the CP concentration of pseudostem and reproductive stem, while pseudostem had greater WSC concentrations than lamina, and reproductive stem was intermediate. As a result, pasture management (such as post-grazing defoliation height) could potentially exert more pronounced influences on herbage WSC:CP by altering the proportion of morphological components in the harvested herbage (Figure 8.7 and 8.8). In Chapter 4, we found that herbage CP concentration decreased, while the WSC concentration increased during both the pasture regrowth and the day. This diurnal pattern of chemical composition resulted in significant increases in herbage WSC:CP, which were consistent and more pronounced than the effect of perennial ryegrass cultivar (Figure 8.7 and 8.8). On the other hand, the timing of daily herbage allocation reduced MUN concentration slightly, but there were small differences in MUN concentration between cultivars (Figure 8.7 and 8.8). In summary, perennial ryegrass cultivar had a similar (compared with the effect of white clover) or less of an effect (compared with the effect of post-grazing defoliation height, regrowth stage and time of day) on predicted NUE than the pasture management investigated in this thesis.

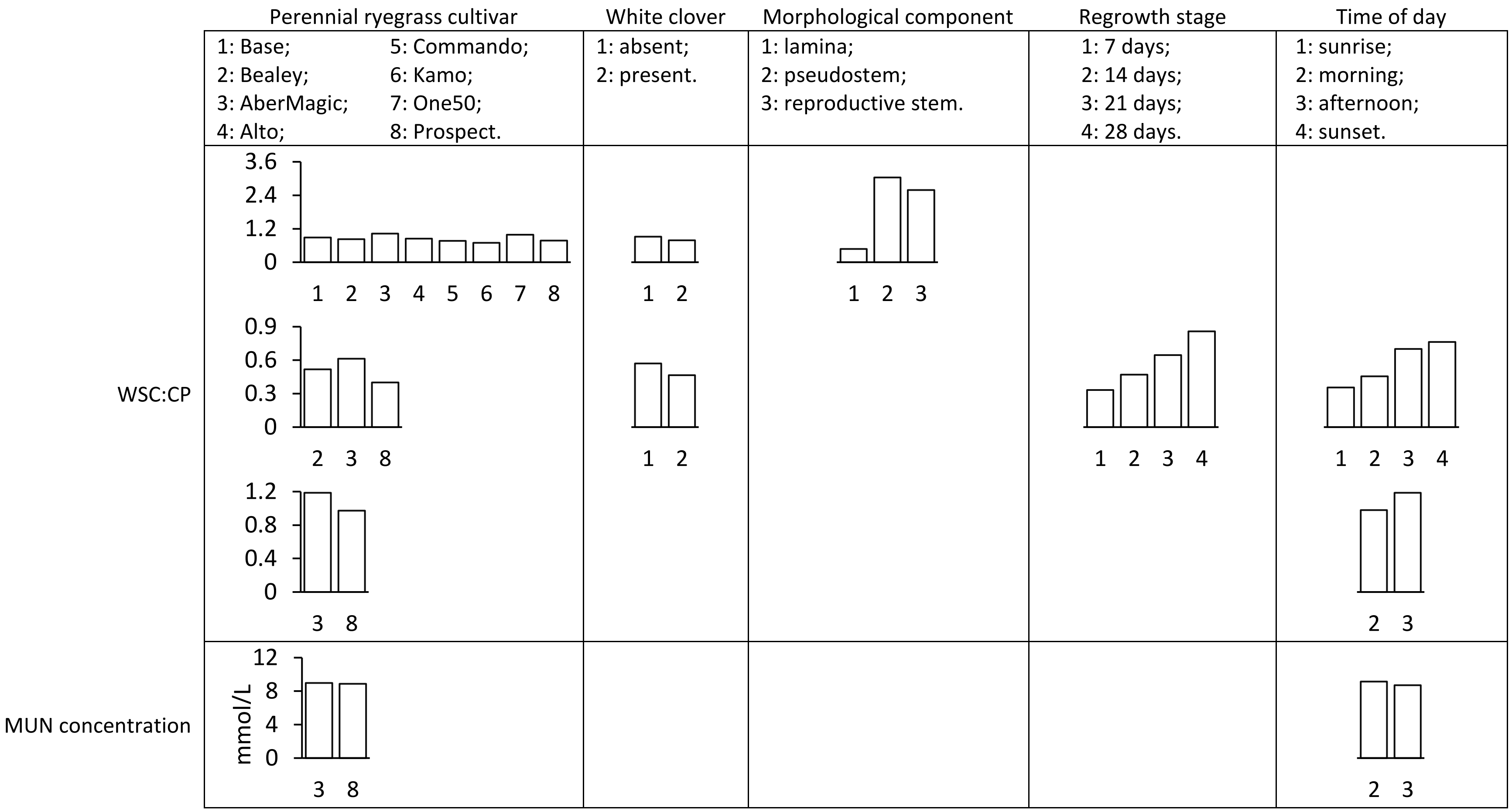


Figure 8.7 Effect of cultivar and management on herbage WSC:CP of perennial ryegrass pastures and MUN concentration.

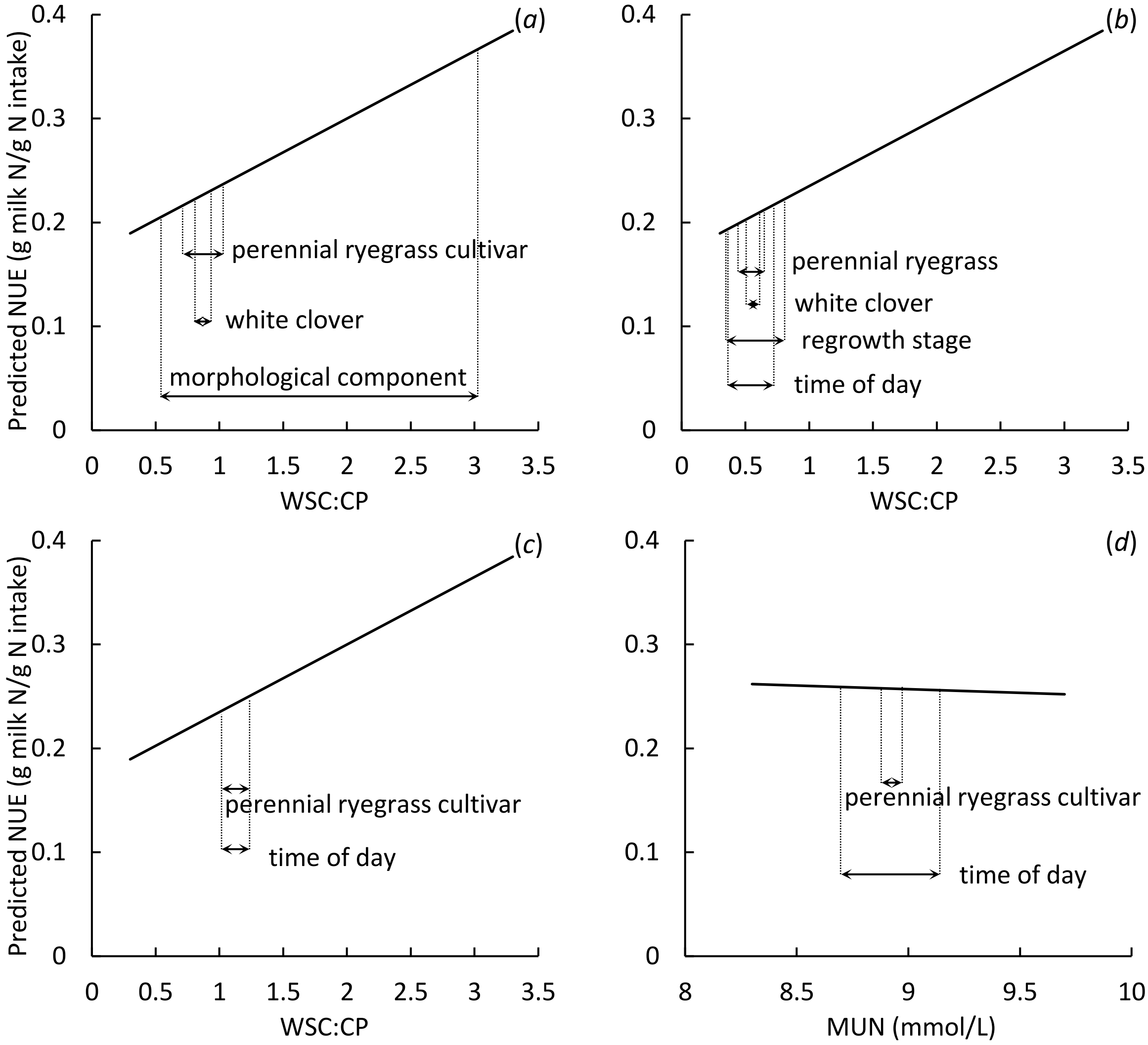


Figure 8.8 Predicted NUE from herbage WSC:CP in Chapter 3 (*a*), 4 (*b*) and 6 (*c*) and MUN in Chapter 7 (*d*). The ranges of perennial ryegrass cultivar effect and pasture management effect on predicted NUE are presented.

8.2 Interactions between cultivar and management

Rasmussen et al. (2009) suggested that any complexity in cultivar × environment and cultivar × management interactions introduced uncertainty in assessing plant traits under uncontrolled field conditions. Cultivar × environment interactions have been well documented previously for herbage DM yield and nutritive value (Lee et al. 2012; Robins & Lovatt 2016). Here, we focused on cultivar × management interaction for the traits related to herbage feeding value of perennial ryegrass cultivars.

8.2.1 White clover

It is a common practice to grow white clover with perennial ryegrass in temperate regions ((Peoples & Baldock 2001). Rossi (2016) reported that no significant interactions were detected between the presence of white clover and perennial ryegrass cultivar on seasonal and annual DM yield with only a few exceptions. Consequently, due to no evidence of cultivar re-ranking, the evaluations of herbage DM yield do not need adjustment to account for the grass-clover interaction.

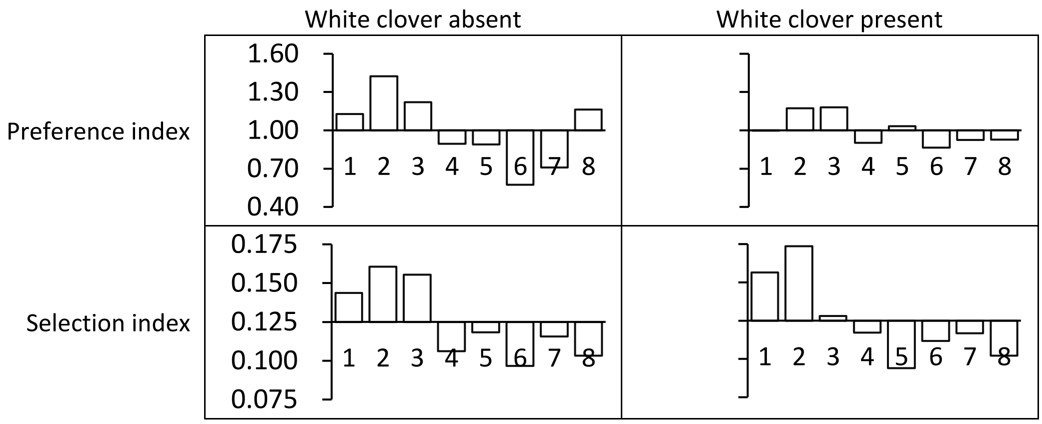
Hypothetically, the potential interaction for feeding value related factors between the presence of white clover and perennial ryegrass cultivar may stem from the different proportions of white clover that perennial ryegrass cultivars can support (Frame & Boyd 1986). However, no significant differences in white clover proportion were found under current conditions (Chapter 3 and 6). Similarly, previous studies in New Zealand (Rossi et al. 2014; Wims et al. 2017) found that perennial ryegrass cultivar ploidy had little effect on the white clover proportion in their swards. This is probably due to the high N fertiliser application rate (325 kg N/ha/year) that suppressed the growth of white clover and led to a low proportion of white clover in all perennial ryegrass cultivars (Labuschagne et al. 2006; Hennessy et al. 2012). The average white clover proportion was around 6% (on a DM basis, above ground level) in Chapter 6 and less than 14% (on a DM basis, 4 cm above ground level) in Chapter 4.

No interactions between white clover and perennial ryegrass cultivar were observed for herbage chemical composition and digestibility at the vegetative stages (Chapter 3 and 6). Likewise, Conaghan et al. (2012) suggested that differences between cultivars in the DMD, Ash, CP and WSC concentrations were largely consistent across different N application rates. At the reproductive stage, there were significant interactions between the perennial ryegrass cultivar and the presence of white clover for herbage WSC and NDF concentrations and DOMD. The reason for these interactions is not clear, but it may be related to the interactions between perennial ryegrass cultivars' reproductive growth and N input (Bahmani et al. 2001b). However, the interaction only occurred at the reproductive stage and the magnitude was small.

White clover interacted with perennial ryegrass to affect the predicted OM and DM potential rumen degradability (Chapter 5). However, feed will theoretically pass through the rumen before it reaches the potential degradability. Therefore, in practice, the degradation of white clover and perennial ryegrass could be considered separately in the rumen without interactions.

Dairy cows showed dietary preference among perennial ryegrass cultivars (Smit et al. 2006) and a partial preference for white clover over perennial ryegrass (Rutter et al. 2004b; Francis et al. 2006). Consequently, the presence of white clover reduced the differences in dietary preference for perennial ryegrass cultivars (Figure 8.9). A similar result was reported that dietary preference between perennial ryegrass with a high and low herbage N concentration was reduced when white clover was offered as another choice (Cosgrove et al. 2002). However, white clover did not result in a complete re-ranking of the dietary preference for cultivars. Tetraploid cultivars (Base and Bealey) and the high-sugar cultivar (AberMagic) were mostly preferred and selected either growing with or without white clover (Figure 8.9).

In summary, under the current conditions when white clover proportion was maintained at a relatively low level (< 7% DM above ground level and < 14% DM 4 cm above ground level), the evaluations of feeding value related factors of perennial ryegrass cultivars were not confounded by the presence of white clover.



1: Base; 2: Bealey; 3: AberMagic; 4: Alto; 5: Commando; 6: Kamo; 7: One50; 8: Prospect.

Figure 8.9 Dietary preference and selection for perennial ryegrass cultivars growing with and without white clover.

8.2.2 Regrowth stage

Interactions between perennial ryegrass cultivar and regrowth stage were found for herbage nutritive value (Chapter 4). Cultivar ranking by the CP and NDF concentration and DOMD changed during pasture regrowth (Figure 8.10). Similarly, Sun et al. (2010) suggested that variations in chemical composition, shear force, shear strength and rumen degradation among perennial ryegrass cultivars could be confounded by the age of regrowth. Additionally, the differences in the WSC concentrations among perennial ryegrass cultivars increased as regrowth proceeded (Figure 8.8). Likewise, Turner et al. (2015) found that the differences in the WSC concentration between high-sugar and normal-sugar cultivars were significantly greater at the 3-leaf stage than the 1.5-leaf stage. As a result, a longer regrowth interval could favour the expression of the high-sugar trait. Therefore, the evaluation of herbage nutritive value of perennial ryegrass cultivars should be conducted at a specified regrowth stage to make cultivars comparable. Also, it is necessary to consider the nutritive value variation during pasture regrowth, in addition to herbage DM yield, to set up a proper regrowth interval for individual cultivars.

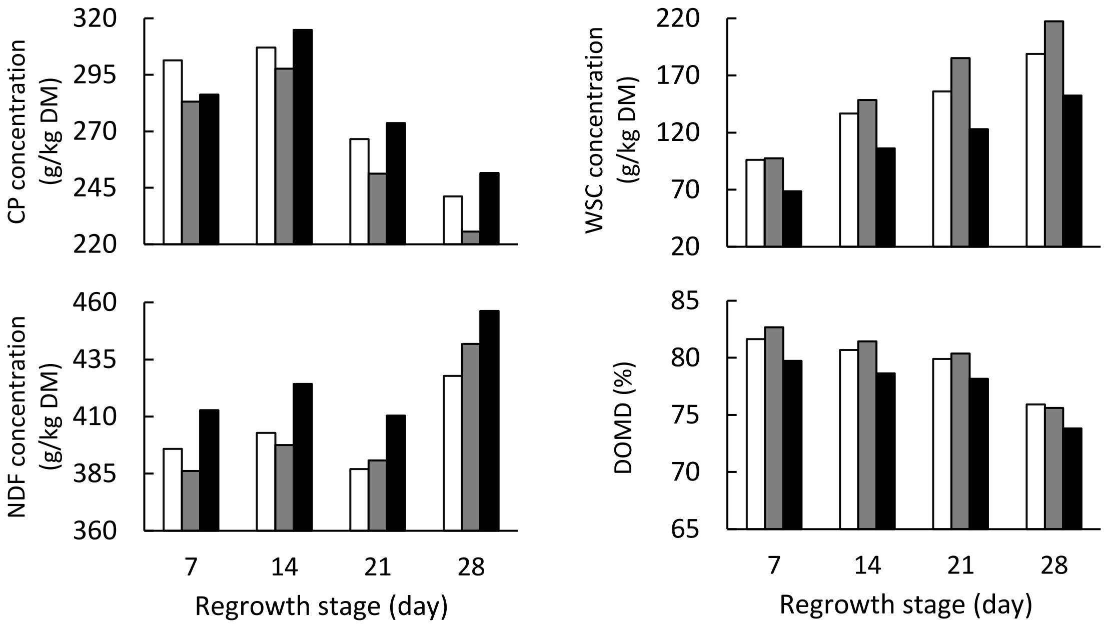


Figure 8.10 Effect of regrowth stage on herbage CP, WSC and NDF concentration and DOMD of Bealey (□), AberMagic (■) and Prospect (■).

8.2.3 Time of day

A tendency for an interaction between perennial ryegrass cultivar and time of day was found in late summer (Chapter 7). Although the AberMagic had a greater overall WSC concentration than Prospect, the difference between cultivars was greater in the morning compared with in the afternoon (Figure 8.11). In other words, the diurnal increase in the WSC concentration was more pronounced in Prospect, compared with AberMagic. As a result, cultivars with a greater WSC concentration diurnal variation might be more suitable for the afternoon herbage allocation system to achieve the benefit of WSC in dairy production. In the evaluations of perennial ryegrass cultivars, it is recommended to sample at different times of day to reflect the herbage nutritive value diurnal variation. Alternatively, at least, the sampling time should be the same for every cultivar and be specified to avoid cultivar × management interactions.

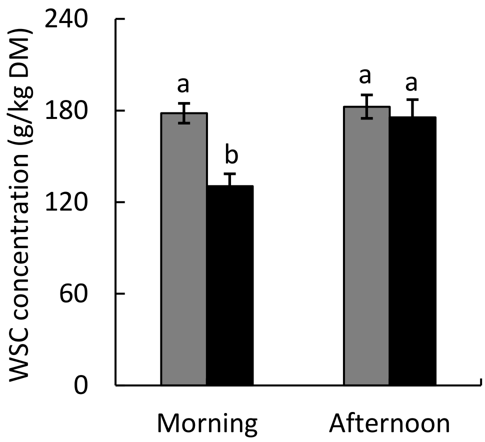


Figure 8.11 Herbage WSC concentration of high-sugar cultivar, AberMagic (■), and normal-sugar cultivar, Prospect (■), harvested in the morning and afternoon. Different letters indicate significantly different means according to Duncan's multiple range test (*P* < 0.05)

8.2.4 Sampling height

Sampling height could also exert an influence on the evaluation of perennial ryegrass cultivars because of the vertical distribution of the nutrients (Delagarde et al. 2000). Although this thesis did not focus on this, there were clues from morphological characteristics in Chapter 3 which showed morphological components differed in chemical composition and digestibility. For example, pseudostem had a greater WSC concentration than lamina consistently. Firstly, the sampling height may change the WSC concentration of the herbage harvested by altering the proportion of pseudostem and dead material included in the herbage. Secondly, the greater variation in the WSC concentration in pseudostem than lamina at the post-flowering vegetative stage (Figure 8.12) suggested that a lower sampling height would lead to greater differences between cultivars. The opposite would happen at the pre-heading vegetative stage (Figure 8.12). Therefore, when samples are taken for the evaluation of herbage nutritive value, the height should be consistent and defined to enable comparisons within the study and with other studies.

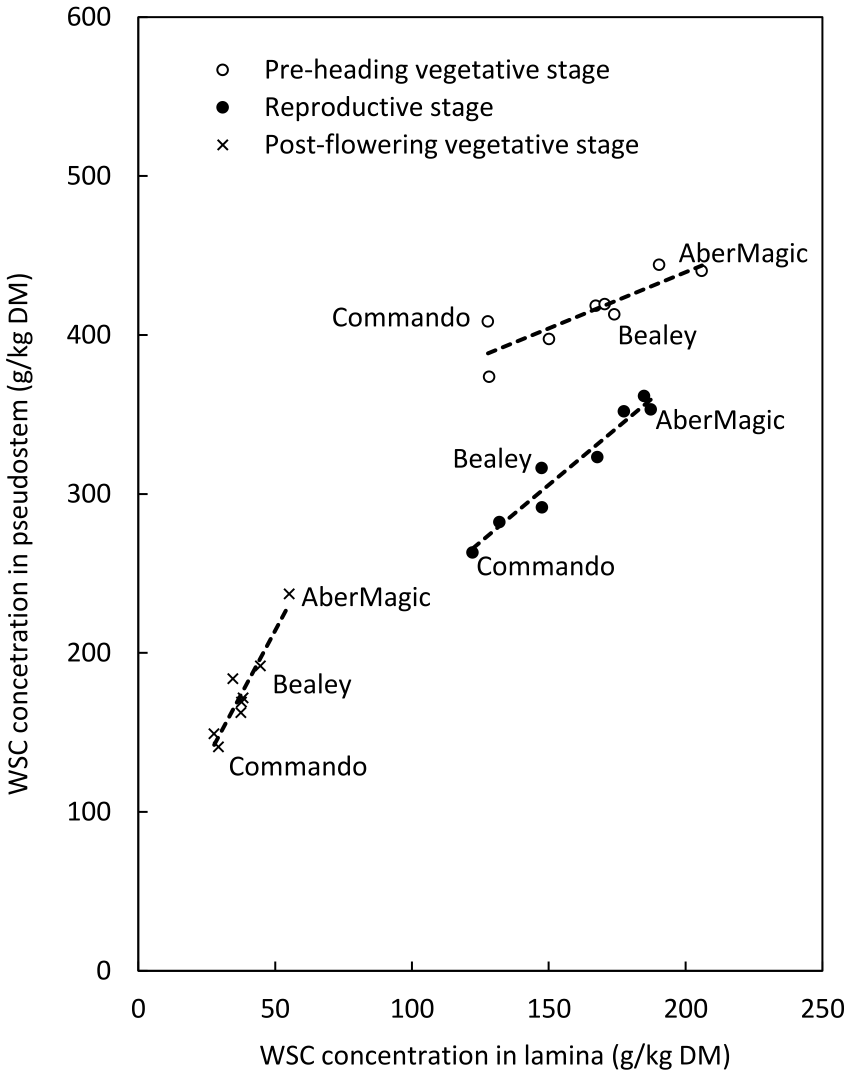


Figure 8.12 Linear relationships between the WSC concentrations in lamina and pseudostem. Data are the means from Chapter 3.

8.3 Overall conclusions

- Among perennial ryegrass cultivars evaluated in this study, there were variations in morphology, nutritive value, rumen degradation, dietary preference and selection. The tetraploid cultivars, Base and Bealey, had a greater lamina proportion. The high-sugar diploid cultivar, AberMagic, had a greater WSC concentration, while Base, Bealey and AberMagic had a greater DOMD. The tetraploid cultivar, Bealey, had a greater degradation rate and a more balanced and synchronised N/energy. Base, Bealey and AberMagic were most preferred and selected by dairy cows.

- The presence of white clover had little effect on morphological characteristics of perennial ryegrass, but it increased herbage CP and ERDP concentrations without an interaction with perennial ryegrass cultivars. The presence of white clover also reduced the differences in dietary preference for perennial ryegrass cultivars, but it did not result in a complete re-ranking of the dietary preference order. Therefore, the presence of white clover had little effect on the evaluations of feeding value related characteristics of perennial ryegrass cultivars.

- During pasture regrowth, herbage WSC and NDF concentration increased, while the CP concentration and DOMD decreased. Interactions between cultivar and regrowth stage indicated that the cultivar ranking by the CP and NDF concentration and DOMD changed during pasture regrowth and the differences in the WSC concentrations among perennial ryegrass cultivars increased as regrowth proceeded.

- During the day, herbage WSC concentration and DOMD increased, while the CP and NDF concentration decreased. The interactions between cultivar and time of day indicated that cultivars with a greater WSC concentration diurnal variation were suitable for the afternoon herbage allocation system to highlight the improvement on milk production and NUE of dairy cows.

- Pasture management exerted pronounced and consistent effects herbage nutritive value, and sometimes outweighed the effects of perennial ryegrass cultivar. Interactions were found between perennial ryegrass cultivar and mangement for herbage nutritive value (the regrowth stage) and dietary preference (the presence of white clover).

8.4 Implications

- Pasture management could potentially be a more effective way to manipulate herbage nutritive value than perennial ryegrass cultivar.

- Evaluations of perennial ryegrass cultivars should be conducted under a defined and consistent condition to exclude the management effects and cultivar × management interactions.

- Pasture management could be adjusted to benefit particular cultivars and cultivars could be selected to fit a certain management system better.

8.5 Suggestions for future research

In this study, the presence of white clover had little effect on the evaluation of perennial ryegrass cultivars, partly due to its low proprtion in the sward under a high N application rate. Therefore, the evaluation of perennial ryegrass cultivars in diverse pastures with more species accounting for greater proportions needs to be further investigated.

It is necessary to study dietary preference and selection of grazing animals in a long-term experiment, in order to explore the implications of dietary preference and selection for pasture persistence and management.

The results suggested that morphology and herbage nutritive value varied at different phenological stages. Future research on milk production over seasons and successive years is required to account for the variations in morphology and nutritive value.

Appendix A

Table A.1 Back-transformed data (g/kg DM).

	CP concentration		CP concentration		
	Lamina	Pseudostem	Lamina	Pseudostem	Reproductive stem
	Pre-heading vegetative stage		*Reproductive stage*		
White clover					
Absent	209	100	211	98	101
Present	215	108	229	107	105
Perennial ryegrass cultivar					
Base	213	101	203	92	94
Bealey	218	107	230	102	102
AberMagic	207	102	213	99	100
Alto	213	104	209	97	103
Commando	218	108	248	123	111
Kamo	216	110	228	105	116
One50	199	97	206	94	98
Prospect	212	104	225	110	101
Mean	213	105	222	104	104

Table A.2 **Parameters in quadratic polynomial regression equations $z = z_0 + ax + by + cx^2 + dy^2 + fxy$ () demonstrating chemical composition and digestibility variation of perennial ryegrass cultivars growing with and without white clover at different times of day (*y*) during pasture regrowth (*x*), *n* = 64.**

	White clover	Perennial ryegrass cultivar	Parameters						R^2	*P* value
			z_0	*a*	*b*	*c*	*d*	*f*		
WSC	Absent	AberMagic	10.86	5.89	137.26	0.00	-17.33	-0.69	0.796	< 0.01
		Bealey	13.33	3.40	172.13	0.07	-42.44	-1.76	0.898	< 0.01
		Prospect	-16.86	5.02	154.36	-0.01	-44.54	-0.47	0.762	< 0.01
	Present	AberMagic	-32.04	10.51	142.51	-0.12	-27.54	-1.39	0.839	< 0.01
		Bealey	-4.38	7.00	134.60	-0.08	-28.13	-0.70	0.743	< 0.01
		Prospect	-17.54	5.67	114.97	-0.05	-13.87	-1.07	0.717	< 0.01
CP	Absent	AberMagic	287.94	3.01	-34.35	-0.19	-5.32	0.10	0.650	< 0.01
		Bealey	317.83	2.53	-75.72	-0.21	21.35	0.99	0.833	< 0.01
		Prospect	262.52	7.34	-39.78	-0.28	27.32	-1.49	0.493	< 0.01
	Present	AberMagic	276.49	5.13	-12.10	-0.21	2.94	-0.94	0.598	< 0.01
		Bealey	318.13	2.32	-47.79	-0.14	12.16	0.07	0.392	< 0.01
		Prospect	266.61	7.40	-22.75	-0.25	7.55	-0.60	0.270	< 0.01
NDF	Absent	AberMagic	416.87	-0.11	-36.56	0.09	15.90	-2.46	0.720	< 0.01
		Bealey	434.46	-2.25	-27.18	0.15	22.03	-3.49	0.741	< 0.01
		Prospect	453.43	-2.57	-17.47	0.17	18.20	-4.12	0.764	< 0.01
	Present	AberMagic	407.37	-4.39	-32.27	0.25	18.73	-2.91	0.826	< 0.01
		Bealey	420.43	-3.55	-46.98	0.19	19.62	-2.44	0.766	< 0.01
		Prospect	423.50	-2.33	-25.87	0.18	14.33	-3.17	0.749	< 0.01
DOMD	Absent	AberMagic	81.09	0.00	3.70	-0.01	-1.17	0.17	0.832	< 0.01
		Bealey	80.64	-0.04	4.50	-0.01	-2.74	0.24	0.853	< 0.01
		Prospect	77.19	0.18	3.66	-0.02	-2.33	0.27	0.852	< 0.01
	Present	AberMagic	79.27	0.31	4.45	-0.02	-2.18	0.15	0.870	< 0.01
		Bealey	78.11	0.27	4.81	-0.02	-2.16	0.15	0.844	< 0.01
		Prospect	77.39	0.19	3.51	-0.02	-1.99	0.20	0.830	< 0.01

Table A.3 Herbage WSC concentration (g/kg DM) of perennial ryegrass growing with and without white clover at different times of day during pasture regrowth.

White clover	Perennial ryegrass cultivar	Regrowth stage (day)	Time of day			
			Sunrise	Morning	Afternoon	Sunset
Absent	Bealey	7	46	81	139	137
		14	66	132	172	196
		21	111	135	183	207
		28	187	188	237	245
	AberMagic	7	67	85	145	146
		14	76	122	189	219
		21	137	155	225	230
		28	188	208	235	276
	Prospect	7	25	46	112	117
		14	51	90	147	156
		21	90	112	176	172
		28	127	144	202	217
Present	Bealey	7	48	71	124	131
		14	66	118	163	182
		21	112	132	179	186
		28	142	139	203	217
	AberMagic	7	47	65	121	129
		14	78	128	180	190
		21	140	153	204	220
		28	181	181	236	238
	Prospect	7	26	48	88	101
		14	48	84	136	145
		21	68	97	147	149
		28	129	107	159	179

Table A.4 Herbage CP concentration (g/kg DM) of perennial ryegrass growing with and without white clover at different times of day during pasture regrowth.

White clover	Perennial ryegrass cultivar	Regrowth stage (day)	Time of day			
			Sunrise	Morning	Afternoon	Sunset
Absent	Bealey	7	330	289	281	284
		14	326	297	291	276
		21	261	256	246	229
		28	217	217	203	193
	AberMagic	7	286	279	278	268
		14	324	291	270	250
		21	256	251	226	224
		28	217	213	215	187
	Prospect	7	294	280	278	275
		14	330	311	289	287
		21	274	263	244	242
		28	254	234	208	200
Present	Bealey	7	324	305	299	296
		14	340	323	302	295
		21	299	284	270	266
		28	269	281	252	246
	AberMagic	7	291	287	289	291
		14	330	317	301	287
		21	279	272	252	248
		28	251	250	234	228
	Prospect	7	301	281	296	288
		14	342	331	307	310
		21	305	297	273	269
		28	270	288	258	259

Table A.5 Herbage NDF concentration (g/kg DM) of perennial ryegrass growing with and without white clover at different times of day during pasture regrowth.

White clover	Perennial ryegrass cultivar	Regrowth stage (day)	Time of day			
			Sunrise	Morning	Afternoon	Sunset
Absent	Bealey	7	409	413	396	399
		14	449	435	392	386
		21	451	406	371	358
		28	475	465	409	396
	AberMagic	7	410	404	388	380
		14	447	437	392	390
		21	437	422	384	374
		28	485	472	407	408
	Prospect	7	434	436	413	414
		14	464	450	406	408
		21	459	437	396	383
		28	514	497	423	410
Present	Bealey	7	406	399	371	368
		14	414	411	369	359
		21	423	385	349	339
		28	449	444	378	374
	AberMagic	7	383	391	360	360
		14	407	397	360	350
		21	418	382	348	348
		28	466	455	396	385
	Prospect	7	417	410	389	384
		14	440	424	391	389
		21	437	408	377	363
		28	481	481	405	396

Table A.6 Herbage DOMD (%) of perennial ryegrass growing with and without white clover at different times of day during pasture regrowth.

White clover	Perennial ryegrass cultivar	Regrowth stage (day)	Time of day			
			Sunrise	Morning	Afternoon	Sunset
Absent	Bealey	7	81.1	81.2	83.1	83.0
		14	77.5	79.3	82.3	82.9
		21	74.8	78.9	82.4	83.5
		28	73.5	74.2	78.7	79.5
	AberMagic	7	81.2	82.1	83.6	84.4
		14	78.2	79.6	82.6	83.2
		21	77.5	78.7	82.2	83.5
		28	73.2	74.2	79.2	79.3
	Prospect	7	78.3	78.8	80.5	81.1
		14	76.1	77.7	81.2	81.0
		21	74.4	76.9	80.5	81.0
		28	70.5	71.5	77.4	78.3
Present	Bealey	7	79.7	80.1	83.0	82.8
		14	78.5	79.4	82.1	82.7
		21	75.9	79.1	82.0	82.3
		28	73.5	73.2	77.9	78.0
	AberMagic	7	81.4	81.0	83.8	84.0
		14	79.3	80.2	83.0	83.6
		21	76.6	79.7	82.5	81.9
		28	72.8	73.0	77.4	77.5
	Prospect	7	78.8	78.7	80.5	81.2
		14	76.0	77.5	80.2	80.2
		21	74.6	77.6	79.9	80.5
		28	71.1	71.0	76.3	76.2

Table A.7 **Parameters in the model ($y = a(1 - e^{-bx})$) demonstrating the changes in sward surface height of perennial ryegrass cultivars growing with and without white clover during grazing time (*x*) at the pre-heading vegetative stage, *n* = 6.**

White clover	Perennial ryegrass cultivar	Parameters		R^2	*P* value
		a	*b*		
Absent	Base	15.96	1.083	0.972	< 0.01
	Bealey	14.92	1.140	0.968	< 0.01
	AberMagic	14.74	1.237	0.972	< 0.01
	Alto	16.28	0.608	0.973	< 0.01
	Commando	16.94	0.721	0.948	< 0.01
	Kamo	17.75	0.547	0.967	< 0.01
	One50	13.15	0.795	0.957	< 0.01
	Prospect	16.39	0.818	0.965	< 0.01
Present	Base	15.21	0.935	0.966	< 0.01
	Bealey	15.85	0.898	0.967	< 0.01
	AberMagic	14.25	1.261	0.958	< 0.01
	Alto	15.53	0.875	0.971	< 0.01
	Commando	17.93	0.786	0.958	< 0.01
	Kamo	17.88	0.819	0.971	< 0.01
	One50	14.99	0.571	0.981	< 0.01
	Prospect	17.25	0.668	0.969	< 0.01

Table A.8 **Parameters in the model ($y = a(1 - e^{-bx})$) demonstrating the changes in sward surface height of perennial ryegrass cultivars growing with and without white clover during grazing time (*x*) at the reproductive stage, *n* = 6.**

White clover	Perennial ryegrass cultivar	Parameters		R^2	*P* value
		a	*b*		
Absent	Base	16.37	1.115	0.993	< 0.01
	Bealey	19.21	1.669	0.996	< 0.01
	AberMagic	16.75	1.355	0.998	< 0.01
	Alto	16.68	1.029	0.996	< 0.01
	Commando	15.36	1.035	0.997	< 0.01
	Kamo	11.40	0.664	0.990	< 0.01
	One50	15.18	0.898	0.996	< 0.01
	Prospect	17.17	1.467	0.992	< 0.01
Present	Base	16.03	1.261	0.993	< 0.01
	Bealey	17.05	1.370	0.993	< 0.01
	AberMagic	16.44	1.438	0.996	< 0.01
	Alto	16.15	1.009	0.996	< 0.01
	Commando	14.58	1.260	0.995	< 0.01
	Kamo	13.75	1.007	0.995	< 0.01
	One50	14.93	1.288	0.990	< 0.01
	Prospect	15.77	1.260	0.993	< 0.01

Table A.9 Parameters in the model ($y = a(1 - e^{-bx})$) demonstrating the changes in sward surface height of perennial ryegrass cultivars growing with and without white clover during grazing time (*x*) at the post-flowering vegetative stage, *n* = 6.

White clover	Perennial ryegrass cultivar	Parameters		R^2	*P* value
		a	*b*		
Absent	Base	12.23	1.224	0.993	< 0.01
	Bealey	13.50	1.374	0.993	< 0.01
	AberMagic	13.95	1.071	0.993	< 0.01
	Alto	12.40	1.147	0.992	< 0.01
	Commando	12.39	1.066	0.994	< 0.01
	Kamo	9.85	0.838	0.960	< 0.01
	One50	11.83	0.783	0.985	< 0.01
	Prospect	12.58	1.291	0.983	< 0.01
Present	Base	12.97	1.039	0.989	< 0.01
	Bealey	14.26	1.349	0.998	< 0.01
	AberMagic	13.39	1.123	0.987	< 0.01
	Alto	12.20	1.058	0.992	< 0.01
	Commando	12.33	1.444	0.983	< 0.01
	Kamo	11.69	1.021	0.993	< 0.01
	One50	14.01	1.257	0.996	< 0.01
	Prospect	12.31	1.152	0.992	< 0.01

References

Abrahamse PA, Tamminge S, Dijkstra J 2009. Effect of daily movement of dairy cattle to fresh grass in morning or afternoon on intake, grazing behaviour, rumen fermentation and milk production. Journal of Agricultural Science 147(6): 721-730.

AFRC 1993. Energy and protein requirements of ruminants: an advisory manual Zaragoza, Spain, Cab International.

Aizimu W, Hodge S, Edwards GR, Dewhurst R, Cheng L 2013. Can milk urea nitrogen differentiate nitrogen use efficiency of lactating cow groups raised on New Zealand pasture? Proceedings of the New Zealand Society of Animal Production 73: 199-201.

Allsop ARC, Nicol AM, Edwards GR 2009. Effect of water soluble carbohydrate concentration of ryegrass on the partial preference of sheep for clover. Proceedings of the New Zealand Society of Animal Production 69: 20-23.

Andrews M, Scholefield D, Abberton MT, McKenzie BA, Hodge S, Raven JA 2007. Use of white clover as an alternative to nitrogen fertiliser for dairy pastures in nitrate vulnerable zones in the UK: productivity, environmental impact and economic considerations. Annals of Applied Biology 151(1): 11-23.

AOAC 1990. Official methods of analysis. Washington, DC., Association of Official Analytical Chemists.

Armstrong CS 1977. 'Grasslands Nui' perennial ryegrass (*Lolium perenne* L.). New Zealand Journal of Experimental Agriculture 5(4): 381-384.

Ayres JF, Nandra KS, Turner AD 1998. A study of the nutritive value of white clover (*Trifolium repens* L.) in relation to different stages of phenological maturity in the primary growth phase in spring. Grass and Forage Science 53(3): 250-259.

Bahmani I, Thom ER, Matthew C, Lemaire G 2001a. Productivity of grazed perennial ryegrass dairy pastures from different ecotypes under nitrogen and irrigation treatments. New Zealand Journal of Agricultural Research 44(2-3): 123-133.

Bahmani I, Thom ER, Matthew C, Lemaire G 2001b. Effect of nitrogen fertilizer on reproductive tiller development in perennial ryegrass. 19th International Grassland Congress. Pp. 39-40.

Barthram GT, Grant SA 1984. Defoliation of ryegrass-dominated swards by sheep. Grass and Forage Science 39(3): 211-219.

Beecher M, Hennessy D, Boland TM, McEvoy M, O'Donovan M, Lewis E 2015. The variation in morphology of perennial ryegrass cultivars throughout the grazing season and effects on organic matter digestibility. Grass and Forage Science 70(1): 19-29.

Belanche A, Lee MRF, Moorby JM, Newbold CJ 2013. Comparison of ryegrass and red clover on the fermentation pattern, microbial community and efficiency of diet utilisation in the rumen simulation technique (Rusitec). Animal Production Science 53(10): 1052-1064.

Berdahl JD, Karn JF, Hendrickson JR 2001. Dry matter yields of cool-season grass monocultures and grass–alfalfa binary mixtures. Agronomy Journal 93(2): 463-467.

Berdahl JD, Karn JF, Hendrickson JR 2004. Nutritive quality of cool-season grass monocultures and binary grass–alfalfa mixtures at late harvest. Agronomy Journal 96(4): 951-955.

Black JL, Kenney PA 1984. Factors affecting diet selection by sheep. 2. Height and density of pasture. Crop and Pasture Science 35(4): 565-578.

Bluett SJ, Thom ER, Clark DA, Macdonald KA, Minneé EMK 2005. Effects of perennial ryegrass infected with either AR1 or wild endophyte on dairy production in the Waikato. New Zealand Journal of Agricultural Research 48(2): 197-212.

Brito AF, Tremblay GF, Bertrand A, Castonguay Y, Bélanger G, Michaud R, Lapierre H, Benchaar C, Petit HV, Ouellet DR 2008. Alfalfa cut at sundown and harvested as baleage improves milk yield of late-lactation dairy cows. Journal of Dairy Science 91(10): 3968-3982.

Brito AF, Tremblay GF, Lapierre H, Bertrand A, Castonguay Y, Bélanger G, Michaud R, Benchaar C, Ouellet DR, Berthiaume R 2009. Alfalfa cut at sundown and harvested as baleage increases bacterial protein synthesis in late-lactation dairy cows. Journal of Dairy Science 92(3): 1092-1107.

Bryant RH, Gregorini P, Edwards GR 2012. Effects of N fertilisation, leaf appearance and time of day on N fractionation and chemical composition of *Lolium perenne* cultivars in spring. Animal Feed Science and Technology 173(3): 210-219.

Bryant RH, Nicol AM, Wilson FE, Sedcole JR 2008. Between-cultivar variability in breaking force of components of perennial ryegrass (*Lolium perenne*). Grass and Forage Science 63(3): 350-359.

Burggraaf V, Waghorn G, Woodward S, Thom E 2008. Effects of condensed tannins in white clover flowers on their digestion *in vitro*. Animal Feed Science and Technology 142(1): 44-58.

Burke D, Pacheco JL, Cosgrove GP 2011. *In vitro* digestion of ryegrasses harvested in the morning and afternoon to manipulate water soluble carbohydrate concentration. Proceedings of the New Zealand Society of Animal Production 71: 229-233.

Burke JL, Waghorn GC, Brookes IM, Attwood GT, Kolver ES, Peterson SW 2000. Formulating total mixed rations from forages-defining the digestion kinetics of contrasting species. Proceedings of the New Zealand Society of Animal Production 60: 9-14.

Burns JC, Mayland HF, Fisher DS 2005. Dry matter intake and digestion of alfalfa harvested at sunset and sunrise. Journal of Animal Science 83(1): 262-270.

Casey IA, Brereton AJ, Laidlaw AS, McGilloway DA 1999. Effects of sheath tube length on leaf development in perennial ryegrass (*Lolium perenne* L.). Annals of Applied Biology 134(2): 251-257.

Cashman PA, McEvoy M, Gilliland TJ, O'Donovan M 2016a. A comparison between cutting and animal grazing for dry-matter yield, quality and tiller density of perennial ryegrass cultivars. Grass and Forage Science 71(1): 112-122.

Cashman PA, Gilliland TJ, McEvoy M, Watson S, O'Donovan M 2016b. Changes in plant morphological expression in 12 perennial ryegrass cultivars following frequent and infrequent cutting management. Journal of Agricultural Science 154(3): 456-471.

Chapman DF, Lee JM, Waghorn GC 2014. Interaction between plant physiology and pasture feeding value: a review. Crop and Pasture Science 65(8): 721-734.

Chapman DF, Tharmaraj J, Agnusdei M, Hill J 2012. Regrowth dynamics and grazing decision rules: further analysis for dairy production systems based on perennial ryegrass (*Lolium perenne* L.) pastures. Grass and Forage Science 67(1): 77-95.

Chapman DF, Lee JM, Rossi L, Cosgrove GP, Stevens DR, Crush JR, King WM, Edwards GR, Popay AJ 2018. Implications of grass-clover interactions in dairy pastures for forage value indexing systems. 1. Context and rationale. New Zealand Journal of Agricultural Research 61(2): 119-146.

Chapman DF, Bryant JR, Olayemi ME, Edwards GR, Thorrold BS, McMillan WH, Kerr GA, Judson G, Cookson T, Moorhead A 2016. An economically based evaluation index for perennial and short-term ryegrasses in New Zealand dairy farm systems. Grass and Forage Science 72(1): 1-21.

Chaves AV, Waghorn GC, Brookes IM, Woodfield DR 2006a. Effect of maturation and initial harvest dates on the nutritive characteristics of ryegrass (*Lolium perenne* L.). Animal Feed Science and Technology 127(3): 293-318.

Chaves AV, Burke JL, Waghorn GC, Brookes IM 2006b. Digestion kinetics of leaf, stem and inflorescence from five species of mature grasses. Journal of the Science of Food and Agriculture 86(5): 816-825.

Chen A, Bryant RH, Edwards GR 2016. Dietary preference of dairy cows for perennial ryegrass cultivars growing with and without white clover. Proceedings of the New Zealand Society of Animal Production 76: 81-86.

Chiy PC, Phillips CJC 1999. Effects of sodium fertiliser on the distribution of trace elements, toxic metals and water-soluble carbohydrates in grass and clover fractions. Journal of the Science of Food and Agriculture 79(14): 2017-2024.

Clark DA, Kanneganti VR 1998. Grazing management systems for dairy cattle. In: Cherney JH, Cherney DJR ed. Grass for Dairy Cattle. Wallingford,UK, CAB International. Pp. 311-334.

Conaghan P, O’Kiely P, Halling MA, O’Mara FP, Nesheim L 2012. Yield and quality response of perennial ryegrass selected for high concentration of water-soluble carbohydrate to nitrogen application rate. Crop Science 52(6): 2839-2851.

Cookson WR, Rowarth JS, Cameron KC 2000. The response of a perennial ryegrass (*Lolium perenne* L.) seed crop to nitrogen fertilizer application in the absence of moisture stress. Grass and Forage Science 55(4): 314-325.

Cosgrove G, Anderson CB, Parsons AJ, Brock JL, Tilbrook JC 2002. Can nitrogen-fertilised ryegrass substitute for white clover? Proceedings of the New Zealand Grassland Association 64: 205-210.

Cosgrove GP 2006. Clover-rich diets and production, behaviour and nutrient use by cows in late lactation. Proceedings of the New Zealand Society of Animal Production 66: 42.

Cosgrove GP, Edwards GR 2007. Control of grazing intake. In: Rattray PV, Brookes IM, Nicol AM ed. Pastures and Supplements for Grazing Animals. Hamilton, New Zealand, New Zealand Society of Animal Production. Pp. 61-80.

Cosgrove GP, Mapp NR, Taylor PS, Harvey BM, Knowler KJ 2014. The chemical composition of high-sugar and control ryegrasses in grazed pasture at different latitudes throughout New Zealand. Proceedings of the New Zealand Grassland Association 76: 169-176.

Cosgrove GP, Burke JL, Death AF, Lane GA, Fraser K, Pacheco D 2006. The effect of clover-rich diets on cows in mid lactation: production, behaviour and nutrient use. Proceedings of the New Zealand Grassland Association 68: 267.

Cosgrove GP, Burke JL, Death AF, Hickey MJ, Pacheco D, Lane GA 2007. Ryegrasses with increased water soluble carbohydrate: evaluating the potential for grazing dairy cows in New Zealand. Proceedings of the New Zealand Grassland Association 69: 179.

Czerkawski JW 1978. Reassessment of efficiency of synthesis of microbial matter in the rumen. Journal of Dairy Science 61(9): 1261-1273.

DairyNZ 2015. Forage value index. Retrieved Febraury 2017 https://www.dairynz.co.nz/media/3361791/forage-value-index-handbook-2015-16.pdf

DairyNZ, NZPBRA 2012. 2011 Forage review. Retrieved February 2017 https://www.dairynz.co.nz/media/178530/2011-forage-review-guide-.pdf

Delagarde R, Peyraud JL, Delaby L, Faverdin P 2000. Vertical distribution of biomass, chemical composition and pepsin–cellulase digestibility in a perennial ryegrass sward: interaction with month of year, regrowth age and time of day. Animal Feed Science and Technology 84(1): 49-68.

Dhanoa MS, France J, López S, Dijkstra J, Lister SJ, Davies DR, Bannink A 1999. Correcting the calculation of extent of degradation to account for particulate matter loss at zero time when applying the polyester bag method. Journal of Animal Science 77(12): 3385-3391.

Di HJ, Cameron KC 2002. The use of a nitrification inhibitor, dicyandiamide (DCD), to decrease nitrate leaching and nitrous oxide emissions in a simulated grazed and irrigated grassland. Soil Use and Management 18(4): 395-403.

Donaghy DJ, Fulkerson WJ 1998. Priority for allocation of water-soluble carbohydrate reserves during regrowth of *Lolium perenne*. Grass and Forage Science 53(3): 211-218.

Easton HS, Baird DB, Cameron NE, Kerr GA, Norriss M, Stewart AV 2001. Perennial ryegrass cultivars: herbage yield in multi-site plot trials. Proceedings of the New Zealand Grassland Association: 183-188.

Edwards GR, Parsons AJ, Bryant RH 2008. Manipulating dietary preference to improve animal performance. Animal Production Science 48(7): 773-779.

Edwards GR, Parsons AJ, Rasmussen S, Bryant RH 2007. High sugar ryegrasses for livestock systems in New Zealand. Proceedings of the New Zealand Grassland Association 69: 161-171.

Egan M, Lynch MB, Hennessy D 2017. Including white clover in nitrogen fertilized perennial ryegrass swards: effects on dry matter intake and milk production of spring calving dairy cows. Journal of Agricultural Science 155(4): 657-668.

Elgersma A, Schlepers H, Nassiri M 2000. Interactions between perennial ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.) under contrasting nitrogen availability: productivity, seasonal patterns of species composition, N_2 fixation, N transfer and N recovery. Plant and Soil 221(2): 281-299.

Enriquez-Hidalgo D, Gilliland TJ, Hennessy D 2016. Herbage and nitrogen yields, fixation and transfer by white clover to companion grasses in grazed swards under different rates of nitrogen fertilization. Grass and Forage Science 71(4): 559-574.

Enriquez-Hidalgo D, Gilliland T, Deighton MH, O'Donovan M, Hennessy D 2014. Milk production and enteric methane emissions by dairy cows grazing fertilized perennial ryegrass pasture with or without inclusion of white clover. Journal of Dairy Science 97(3): 1400-1412.

Evans DR, Humphreys MO, Williams TA 1996. Forage yield and quality interactions between white clover and contrasting ryegrass varieties in grazed swards. Journal of Agricultural Science 126(3): 295-299.

Fè D, Pedersen MG, Jensen CS, Jensen J 2015. Genetic and environmental variation in a commercial breeding program of perennial ryegrass. Crop Science 55(2): 631-640.

Fisher DS, Mayland HF, Burns JC 1999. Variation in ruminants' preference for tall fescue hays cut either at sundown or at sunup. Journal of Animal Science 77(3): 762-768.

Frame J, Boyd AG 1986. Effect of cultivar and seed rate of perennial ryegrass and strategic fertilizer nitrogen on the productivity of grass/white clover swards. Grass and Forage Science 41(4): 359-366.

Frame J, Charlton JFL, Laidlaw AS 1998. Temperate forage legumes. Wallingford, UK, Cab International.

Francis SA, Chapman DF, Doyle PT, Leury BJ 2006. Dietary preferences of cows offered choices between white clover and 'high sugar' and 'typical' perennial ryegrass cultivars. Animal Production Science 46(12): 1579-1587.

Fulkerson WJ, Donaghy DJ 2001. Plant-soluble carbohydrate reserves and senescence-key criteria for developing an effective grazing management system for ryegrass-based pastures: a review. Animal Production Science 41(2): 261-275.

Fulkerson WJ, Slack K, Hennessy DW, Hough GM 1998. Nutrients in ryegrass (*Lolium* spp.), white clover (*Trifolium repens*) and kikuyu (*Pennisetum clandestinum*) pastures in relation to season and stage of regrowth in a subtropical environment. Animal Production Science 38(3): 227-240.

Gallagher JA, Turner LB, Cairns AJ, Farrell M, Lovatt JA, Skøt K, Armstead IP, Humphreys MO, Roldan-Ruiz I 2015. Genetic differentiation in response to selection for water-soluble carbohydrate content in perennial ryegrass (*Lolium perenne* L.). BioEnergy Research 8(1): 77-90.

Gallagher RT, White EP, Mortimer PH 1981. Ryegrass staggers: isolation of potent neurotoxins lolitrem A and lolitrem B from staggers-producing pastures. New Zealand Veterinary Journal 29(10): 189-190.

Ganche E, O'Donovan M, Delaby L, Boland TM, Kennedy E 2015. Does post-grazing sward height influence sward characteristics, seasonal herbage dry-matter production and herbage quality? Grass and Forage Science 70(1): 130-143.

Gastal F, Lemaire G 2002. N uptake and distribution in crops: an agronomical and ecophysiological perspective. Journal of Experimental Botany 53(370): 789-799.

Gerber L, Gerber JJ 2000. Production and physiological responses of Italian ryegrass and white clover grown in monocultures and mixed stands. African Journal of Range and Forage Science 17(1-3): 101-110.

Gilliland TJ, Mann RL 2000. Effect of sward cutting management on the relative performance of perennial ryegrass varieties. Journal of Agricultural Science 135(2): 113-122.

Gilliland TJ, Barrett PD, Mann RL, Agnew RE, Fearon AM 2002. Canopy morphology and nutritional quality traits as potential grazing value indicators for *Lolium perenne* varieties. Journal of Agricultural Science 139(3): 257-273.

Gowen N, O'Donovan M, Casey I, Rath M, Delaby L, Stakelum G 2003. The effect of grass cultivars differing in heading date and ploidy on the performance and dry matter intake of spring calving dairy cows at pasture. Animal Research 52(4): 321-336.

Green JO, Corrall AJ, Terry RA 1971. Grass species and varieties. Relationships between stage of growth, yield and forage quality. Technical Report 8. Hurley Berks, UK, Grassland Research Institute.

Gregorini P 2012. Diurnal grazing pattern: its physiological basis and strategic management. Animal Production Science 52(7): 416-430.

Gregorini P, Soder KJ, Sanderson MA, Ziegler GR 2009. Toughness, particle size and chemical composition of meadow fescue (*Festuca pratensis* Hud.) herbage as affected by time of day. Animal Feed Science and Technology 151(3): 330-336.

Gregorini P, Eirin M, Refi R, Ursino M, Ansin OE, Gunter SA 2006. Timing of herbage allocation in strip grazing: Effects on grazing pattern and performance of beef heifers. Journal of Animal Science 84(7): 1943-1950.

Halling MA, Longland AC, Martens S, Nesheim L, O'Kiely P 2005. Water soluble carbohydrate content of two cultivars of perennial ryegrass (*Lolium perenne*) at eight European sites. 20th International Grassland Congress. Pp. 131.

Hammond KJ, Hoskin SO, Burke JL, Waghorn GC, Koolaard JP, Muetzel S 2011. Effects of feeding fresh white clover (*Trifolium repens*) or perennial ryegrass (*Lolium perenne*) on enteric methane emissions from sheep. Animal Feed Science and Technology 166: 398-404.

Harmer M, Stewart AV, Woodfield DR 2016. Genetic gain in perennial ryegrass forage yield in Australia and New Zealand. Journal of New Zealand Grasslands 78: 133-138.

Harris SL, Auldist MJ, Clark DA, Jansen EB 1998. Effects of white clover content in the diet on herbage intake, milk production and milk composition of New Zealand dairy cows housed indoors. Journal of Dairy Research 65(3): 389-400.

Heeren JAH, Podesta SC, Hatew B, Klop G, Van Laar H, Bannink A, Warner D, De Jonge LH, Dijkstra J 2014. Rumen degradation characteristics of ryegrass herbage and ryegrass silage are affected by interactions between stage of maturity and nitrogen fertilisation rate. Animal Production Science 54(9): 1263-1267.

Hennessy D, Enriquez-Hidalgo D, O'Donovan M, Gilliland T 2012. Effect of N fertilizer application rate on herbage production and sward clover contents in grazed grass clover plots. 24th General Meeting of the European Grassland Federation. Pp. 124-127.

Hodgson J 1979. Nomenclature and definitions in grazing studies. Grass and Forage Science 34(1): 11-17.

Hodgson J 1985. The control of herbage intake in the grazing ruminant. Proceedings of the Nutrition Society 44(2): 339-346.

Hoffman PC, Sievert SJ, Shaver RD, Welch DA, Combs DK 1993. *In situ* dry matter, protein, and fiber degradation of perennial forages. Journal of Dairy Science 76(9): 2632-2643.

Holmes CW, Wilson GF, Mackenzie DDS, Flux DS, Brookes IM, Davey AWF 1987. Milk production from pasture. Wellington, New Zealand, Butterworths of New Zealand Ltd.

Holt DA, Hilst AR 1969. Daily variation in carbohydrate content of selected forage crops. Agronomy Journal 61(2): 239-242.

Hristov AN 1998. Nitrogen fractions and *in sacco* dry matter and crude protein degradability of fresh and frozen alfalfa. Animal Feed Science and Technology 71(3): 351-355.

Hristov AN, Ropp JK, Grandeen KL, Abedi S, Etter RP, Melgar A, Foley AE 2005. Effect of carbohydrate source on ammonia utilization in lactating dairy cows. Journal of Animal Science 83(2): 408-421.

Humphreys J, O'Kiely P 2006. Amount and quality of grass harvested for first-cut silage for differing spring-grazing frequencies of two mixtures of perennial ryegrass cultivars with contrasting heading date. Grass and Forage Science 61(1): 77-88.

Humphreys M, Feuerstein U, Vandewalle M, Baert J 2010. Ryegrasses. In: Boller B, Posselt UK, Veronesi F ed. Fodder Crops and Amenity Grasses, Springer. Pp. 211-260.

Humphreys MO 1989a. Water-soluble carbohydrates in perennial ryegrass breeding. III. Relationships with herbage production, digestibility and crude protein content. Grass and Forage Science 44(4): 423-430.

Humphreys MO 1989b. Water-soluble carbohydrates in perennial ryegrass breeding. I. Genetic differences among cultivars and hybrid progeny grown as spaced plants. Grass and Forage Science 44(2): 231-236.

Humphreys MO 1989c. Water-soluble carbohydrates in perennial ryegrass breeding. II. Cultivar and hybrid progeny performance in cut plots. Grass and Forage Science 44(2): 237-244.

Humphreys MO 2005. Genetic improvement of forage crops-past, present and future. Journal of Agricultural Science 143(6): 441-448.

Hunt WF, Easton HS 1989. Fifty years of ryegrass research in New Zealand. Proceedings of the New Zealand Grassland Association 50(1): 1-23.

Huntington GB, Burns JC 2007. Afternoon harvest increases readily fermentable carbohydrate concentration and voluntary intake of gamagrass and switchgrass baleage by beef steers. Journal of Animal Science 85(1): 276-284.

Hurley G, O'Donovan M, Gilliland TJ 2009. Effect of spring defoliation pattern on the mid-season production and morphology of swards of perennial ryegrass cultivars of different maturity. Grass and Forage Science 64(1): 80-91.

Hyslop MG, Fraser TJ, Smith DR, Knight TL, Slay MWA, Moffat CA, Peterson SW 2000. Liveweight gain of young sheep grazing tall fescue or perennial ryegrass swards of different white clover content. Proceedings of the New Zealand Society of Animal Production 60: 51-54.

Illius AW, Clark DA, Hodgson J 1992. Discrimination and patch choice by sheep grazing grass-clover swards. Journal of Animal Ecology 61(1): 183-194.

Jaurena G, Moorby JM, Davies DR 2005. Efficiency of microbial protein synthesis on red clover and ryegrass silages supplemented with barley by rumen simulation technique (Rusitec). Animal Feed Science and Technology 118(1): 79-91.

Jay M, Morad M 2007. Crying over spilt milk: A critical assessment of the ecological modernization of New Zealand's dairy industry. Society and Natural Resources 20(5): 469-478.

Johnson LJ, de Bonth ACM, Briggs LR, Caradus JR, Finch SC, Fleetwood DJ, Fletcher LR, Hume DE, Johnson RD, Popay AJ 2013. The exploitation of epichloae endophytes for agricultural benefit. Fungal Diversity 60(1): 171-188.

Jones DIH, Hayward MV 1975. The effect of pepsin pretreatment of herbage on the prediction of dry matter digestibility from solubility in fungal cellulase solutions. Journal of the Science of Food and Agriculture 26(5): 711-718.

Kauffman AJ, St-Pierre NR 2001. The relationship of milk urea nitrogen to urine nitrogen excretion in Holstein and Jersey cows. Journal of Dairy Science 84(10): 2284-2294.

Kerr GA, Chapman DF, Thom ER, Matthew C, Van Der Linden A, Baird DB, Johnston E, Corkran JR 2012. Evaluating perennial ryegrass cultivars: improving testing. Proceedings of the New Zealand Grassland Association 74: 127-136.

Kilgour R, Dalton C 1984. Livestock Behaviour. Great Britain, Granada Publishing Ltd.

Kokko C, Soder KJ, Brito AF, Hovey RC, Berthiaume R 2013. Effect of time of cutting and maceration on nutrient flow, microbial protein synthesis, and digestibility in dual-flow continuous culture. Journal of Animal Science 91(4): 1765-1774.

Kunelius HT, Narasimhalu P 1983. Yields and quality of Italian and Westerwolds ryegrasses, red clover, alfalfa, birdsfoot trefoil, and Persian clover grown in monocultures and ryegrass-legume mixtures. Canadian Journal of Plant Science 63(2): 437-442.

Kyriazakis I 1996. Nutritional choices in farm animals: to eat or what to eat? In: Forbes J, Lawrence R, Rodway M ed. Animal Choices. Pp. 55– 65.

Labuschagne J, Hardy MB, Agenbag GA 2006. The effects of strategic nitrogen fertiliser application during the cool season on perennial ryegrass-white clover pastures in the Western Cape Province 3. Clover content. South African Journal of Plant and Soil 23(4): 269-276.

Laca EA, Ungar ED, Seligman N, Demment MW 1992. Effects of sward height and bulk density on bite dimensions of cattle grazing homogeneous swards. Grass and Forage Science 47(1): 91-102.

Laidlaw AS 2004. Effect of heading date of perennial ryegrass cultivars on tillering and tiller development in spring and summer. Grass and Forage Science 59(3): 240-249.

Laidlaw AS 2005. The relationship between tiller appearance in spring and contribution to dry-matter yield in perennial ryegrass (*Lolium perenne* L.) cultivars differing in heading date. Grass and Forage Science 60(2): 200-209.

Laidlaw AS, Teuber N 2001. Temperate forage grass-legume mixtures: advances and perspectives. 19th International Grassland Congress. Pp. 85-92.

Langer RHM 1990. Pastures, their ecology and management, Oxford University Press

Ledgard SF, Crush JR, Penno JW 1998. Environmental impacts of different nitrogen inputs on dairy farms and implications for the Resource Management Act of New Zealand. Environmental Pollution 102(1): 515-519.

Lee JM, Matthew C, Thom ER, Chapman DF 2012. Perennial ryegrass breeding in New Zealand: a dairy industry perspective. Crop and Pasture Science 63(2): 107-127.

Lee MRF, Harris LJ, Moorby JM, Humphreys MO, Theodorou MK, MacRae JC, Scollan ND 2002. Rumen metabolism and nitrogen flow to the small intestine in steers offered *Lolium perenne* containing different levels of water-soluble carbohydrate. Animal Science 74(3): 587-596.

LIC, DairyNZ 2016. New Zealand dairy statistics 2015-16. Retrieved February 2017 https://www.dairynz.co.nz/media/5416078/nz-dairy-statistics-2015-16.pdf

López S, Hovell FDD, Manyuchi B, Smart RI 1995. Comparison of sample preparation methods for the determination of the rumen degradation characteristics of fresh and ensiled forages by the nylon bag technique. Animal Science 60(3): 439-450.

Lovett DK, McGilloway D, Bortolozzo A, Hawkins M, Callan J, Flynn B, O'Mara FP 2006. *In vitro* fermentation patterns and methane production as influenced by cultivar and season of harvest of *Lolium perenne* L. Grass and Forage Science 61(1): 9-21.

MAFF 1986. The analysis of agricultural materials. 3rd ed. London, Her Majesty's Stationery Office.

Mambrini M, Peyraud JL, Hetault H, Stephant A, Texier M 1994. Mean retention time in digestive tract and digestion of fresh perennial ryegrass by lactating dairy cows: influence of grass maturity and comparison with a maize silage diet. Reproduction Nutrition Development 34(1): 9-23.

Manly BFJ 1974. A model for certain types of selection experiments. Biometrics 30(2): 281-294.

Mayland HF, Shewmaker GE, Harrison PA, Chatterton NJ 2000. Nonstructural carbohydrates in tall fescue cultivars: Relationship to animal preference. Agronomy Journal 92(6): 1203-1206.

McDonagh J, Gilliland TJ, McEvoy M, Delaby L, O'Donovan M 2017. Nitrogen and white clover impacts on the management of perennial ryegrass-clover swards for grazing cattle. Journal of Agricultural Science 155(9): 1381-1393.

McEvoy M, O'Donovan M, Shalloo L 2011. Development and application of an economic ranking index for perennial ryegrass cultivars. Journal of Dairy Science 94(3): 1627-1639.

McGrath D 1988. Seasonal variation in the water-soluble carbohydrates of perennial and Italian ryegrass under cutting conditions. Irish Journal of Agricultural Research 12(2): 131-139.

McGrath S, Hodkinson TR, Frohlich A, Grant J, Barth S 2014. Seasonal and genetic variations in water-soluble carbohydrates and other quality traits in ecotypes and cultivars of perennial ryegrass (*Lolium perenne* L.). Plant Genetic Resources 12(2): 236-247.

Miller LA, Moorby JM, Davies DR, Humphreys MO, Scollan ND, MacRae JC, Theodorou MK 2001. Increased concentration of water-soluble carbohydrate in perennial ryegrass (*Lolium perenne* L.): milk production from late-lactation dairy cows. Grass and Forage Science 56(4): 383-394.

Min BR, McNabb WC, Barry TN, Peters JS 2000. Solubilization and degradation of ribulose-1, 5-bisphosphate carboxylase/oxygenase (EC 4.1. 1.39; Rubisco) protein from white clover (*Trifolium repens*) and Lotus corniculatus by rumen microorganisms and the effect of condensed tannins on these processes. Journal of Agricultural Science 134(3): 305-317.

Min BR, Barry TN, Attwood GT, McNabb WC 2003. The effect of condensed tannins on the nutrition and health of ruminants fed fresh temperate forages: a review. Animal Feed Science and Technology 106(1): 3-19.

Ministry for the Environment 2012. Environmental reporting. Retrieved February 2017 https://data.mfe.govt.nz/data/category/environmental-reporting/land/land-use/

Minson DJ 1960. The digestibility of grass species and varieties. 8th International Grassland Congress.

Moharrery A, Hvelplund T, Weisbjerg MR 2009. Effect of forage type, harvesting time and exogenous enzyme application on degradation characteristics measured using in vitro technique. Animal Feed Science and Technology 153(3): 178-192.

Moorby JM, Evans RT, Scollan ND, MacRae JC, Theodorou MK 2006. Increased concentration of water-soluble carbohydrate in perennial ryegrass (*Lolium perenne* L.). Evaluation in dairy cows in early lactation. Grass and Forage Science 61(1): 52-59.

Moorby JM, Lee MRF, Davies DR, Kim EJ, Nute GR, Ellis NM, Scollan ND 2009. Assessment of dietary ratios of red clover and grass silages on milk production and milk quality in dairy cows. Journal of Dairy Science 92(3): 1148-1160.

Morin C, Bélanger G, Tremblay GF, Bertrand A, Castonguay Y, Drapeau R, Michaud R, Berthiaume R, Allard G 2011. Diurnal variations of nonstructural carbohydrates and nutritive value in alfalfa. Crop Science 51(3): 1297-1306.

Morvan-Bertrand A, Ernstsen A, Lindgård B, Koshioka M, Le Saos J, Boucaud J, Prud'homme M, Junttila O 2001. Endogenous gibberellins in *Lolium perenne* and influence of defoliation on their contents in elongating leaf bases and in leaf sheaths. Physiologia Plantarum 111(2): 225-231.

Nicol AM, Edwards GR 2011. Why is clover better than ryegrass. Proceedings of the New Zealand Grassland Association 71: 71-78.

Niderkorn V, Baumont R, Le Morvan A, Macheboeuf D 2011. Occurrence of associative effects between grasses and legumes in binary mixtures on *in vitro* rumen fermentation characteristics. Journal of Animal Science 89(4): 1138-1145.

Niderkorn V, Mueller-Harvey I, Le Morvan A, Aufrère J 2012. Synergistic effects of mixing cocksfoot and sainfoin on *in vitro* rumen fermentation. Role of condensed tannins. Animal Feed Science and Technology 178(1): 48-56.

Nocek JE, Russell JB 1988. Protein and energy as an integrated system. Relationship of ruminal protein and carbohydrate availability to microbial synthesis and milk production. Journal of Dairy Science 71(8): 2070-2107.

Orr R, Rutter S, Penning P, Rook A 2001a. Matching grass supply to grazing patterns for dairy cows. Grass and Forage Science 56(4): 352-361.

Orr RJ, Penning PD, Harvey A, Champion RA 1997. Diurnal patterns of intake rate by sheep grazing monocultures of ryegrass or white clover. Applied Animal Behaviour Science 52(1): 65-77.

Orr RJ, Rutter SM, Penning PD, Rook AJ 2001b. Matching grass supply to grazing patterns for dairy cows. Grass and Forage Science 56(4): 352-361.

Orr RJ, Cook JE, Champion RA, Rook AJ 2004. Relationships between morphological and chemical characteristics of perennial ryegrass varieties and intake by sheep under continuous stocking management. Grass and Forage Science 59(4): 389-398.

Ørskov ER, McDonald I 1979. The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. Journal of Agricultural Science 92(2): 499-503.

Parsons AJ, Rasmussen S, Xue H, Newman JA, Anderson CB, Cosgrove GP 2004. Some 'high sugar grasses' don’t like it hot. Proceedings of the New Zealand Grassland Association 66: 265-271.

Paynel F, Lesuffleur F, Bigot J, Diquélou S, Cliquet J 2008. A study of ^{15}N transfer between legumes and grasses. Agronomy for Sustainable Development 28(2): 281-290.

Pelletier S, Tremblay GF, Lafrenière C, Bertrand A, Bélanger G, Castonguay Y, Rowsell J 2009. Nonstructural carbohydrate concentrations in timothy as affected by N fertilization, stage of development, and time of cutting. Agronomy Journal 101(6): 1372-1380.

Peoples MB, Baldock JA 2001. Nitrogen dynamics of pastures: nitrogen fixation inputs, the impact of legumes on soil nitrogen fertility, and the contributions of fixed nitrogen to Australian farming systems. Animal Production Science 41(3): 327-346.

Phillips C 2002. Cattle behaviour & welfare. Malden, USA, Blackwell Science Ltd.

Phillips CJC, James NL 1998. The effects of including white clover in perennial ryegrass swards and the height of mixed swards on the milk production, sward selection and ingestive behaviour of dairy cows. Animal Science 67(2): 195-202.

Poppi DP, Minson DJ, Ternouth JH 1981. Studies of cattle and sheep eating leaf and stem fractions of grasses. 1. The voluntary intake, digestibility and retention time in the reticulo-rumen. Crop and Pasture Science 32(1): 99-108.

Pulido RG, Ruiz-Albarrán M, Balocchi OA, Nannig P, Wittwer F 2015. Effect of timing of pasture allocation on production, behavior, rumen function, and metabolism of early lactating dairy cows during autumn. Livestock Science 177: 43-51.

Purcell PJ, O'Brien M, Navarro-Villa A, Boland TM, McEvoy M, Grogan D, O'Kiely P 2012. *In vitro* rumen methane output of perennial ryegrass varieties and perennial grass species harvested throughout the growing season. Grass and Forage Science 67(2): 280-298.

Purwin C, Stanek M, Lipinski K, Wierzbowska J, Nogalska A, Fijalkowska M 2016. Effect of a harvest time and cultivar on the chemical composition and in vitro ruminal dry matter degradability of perennial ryegrass (*Lolium perenne* L.). Journal of Elementology 21(3): 811-822.

Rasmussen S, Parsons AJ, Xue H, Newman JA 2009. High sugar grasses-harnessing the benefits of new cultivars through growth management. Proceedings of the New Zealand Grassland Association 71: 167-175.

Reid D, Strachan NH 1974. The effects of a wide range of nitrogen rates on some chemical constituents of the herbage from perennial ryegrass swards with and without white clover. Journal of Agricultural Science 83(3): 393-401.

Ribeiro Filho HMN, Delagarde R, Peyraud JL 2005. Herbage intake and milk yield of dairy cows grazing perennial ryegrass swards or white clover/perennial ryegrass swards at low-and medium-herbage allowances. Animal Feed Science and Technology 119(1): 13-27.

Robins JG, Lovatt JA 2016. Cultivar by environment effects of perennial ryegrass cultivars selected for high water soluble carbohydrates managed under differing precipitation levels. Euphytica 208(3): 571-581.

Roegiers P, Reheul D, van Bogaert G 1988. The persistence of tetraploid perennial ryegrass in a mixture with diploid perennial ryegrass. Journal of Agronomy and Crop Science 161(1): 40-44.

Rogers A, Fischer BU, Bryant J, Frehner M, Blum H, Raines CA, Long SP 1998. Acclimation of photosynthesis to elevated CO_2 under low-nitrogen nutrition is affected by the capacity for assimilate utilization. Perennial ryegrass under free-air CO_2 enrichment. Plant Physiology 118(2): 683-689.

Rogers GL, Porter RHD, Robinson I 1982. Comparison of perennial ryegrass and white clover for milk production. In: Macmillan KL, Taufa VK ed. Dairy Production from Pasture. Hamilton, New Zealand, New Zealand Society of Animal Production. Pp. 213-214.

Rossi L, McDonagh J, McEvoy M, O'Donovan M, Lee JM, Chapman DF, Edwards GR 2014. Implications of species and management interactions for ranking perennial ryegrass (*Lolium perenne* L.) cultivars: a progress report from two hemispheres. In: Roche JR ed. 6th Australasian Dairy Science Symposium. Pp. 259-262.

Rossi LI 2016. Interactions between pasture species and management and their implications for evaluating perennial ryegrass (*Lolium perenne* L.) cultivars in dairy systems. Unpublished thesis, Lincoln University, New Zealand.

Rutter SM 2006. Diet preference for grass and legumes in free-ranging domestic sheep and cattle: current theory and future application. Applied Animal Behaviour Science 97(1): 17-35.

Rutter SM, Orr RJ, Yarrow NH, Champion RA 2004a. Dietary preference of dairy heifers grazing ryegrass and white clover, with and without an anti-bloat treatment. Applied Animal Behaviour Science 85(1): 1-10.

Rutter SM, Orr RJ, Yarrow NH, Champion RA 2004b. Dietary preference of dairy cows grazing ryegrass and white clover. Journal of Dairy Science 87(5): 1317-1324.

Rutter SM, Orr RJ, Penning PD, Yarrow NH, Champion RA 2002. Ingestive behaviour of heifers grazing monocultures of ryegrass or white clover. Applied Animal Behaviour Science 76(1): 1-9.

Ryle GJA, Powell CE, Gordon AJ 1985. Defoliation in white clover: regrowth, photosynthesis and N_2 fixation. Annals of Botany 56(1): 9-18.

Sauvé AK, Huntington GB, Burns JC 2009. Effects of total nonstructural carbohydrates and nitrogen balance on voluntary intake of goats and digestibility of gamagrass hay harvested at sunrise and sunset. Animal Feed Science and Technology 148(2): 93-106.

Shewmaker GE, Mayland HF, Roberts CA, Harrison PA, Chatterton NJ, Sleper DA 2006. Daily carbohydrate accumulation in eight tall fescue cultivars. Grass and Forage Science 61(4): 413-421.

Sinclair LA, Garnsworth PC, Newbold JR, Buttery PJ 1993. Effect of synchronizing the rate of dietary energy and nitrogen release on rumen fermentation and microbial protein synthesis in sheep. Journal of Agricultural Science 120(2): 251-263.

Smit HJ, Tamminga S, Elgersma A 2006. Dairy cattle grazing preference among six cultivars of perennial ryegrass. Agronomy Journal 98(5): 1213-1220.

Smit HJ, Tas BM, Taweel HZ, Elgersma A 2005. Sward characteristics important for intake in six *Lolium perenne* varieties. Grass and Forage Science 60(2): 128-135.

Smith KF, Simpson RJ, Culvenor RA, Humphreys MO, Prud'Homme MP, Oram RN 2001. The effects of ploidy and a phenotype conferring a high water-soluble carbohydrate concentration on carbohydrate accumulation, nutritive value and morphology of perennial ryegrass (*Lolium perenne* L.). Journal of Agricultural Science 136(1): 65-74.

Smith KF, Simpson RJ, Oram RN, Lowe KF, Kelly KB, Evans PM, Humphreys MO 1999. Seasonal variation in the herbage yield and nutritive value of perennial ryegrass (*Lolium perenne* L.) cultivars with high or normal herbage water-soluble carbohydrate concentrations grown in three contrasting Australian dairy environments. Animal Production Science 38(8): 821-830.

Solomon JKQ, Macoon B, Lang DJ, Vann RC, Ward S 2014. Cattle grazing preference among tetraploid and diploid annual ryegrass cultivars. Crop Science 54(1): 430-438.

Staerfl SM, Amelchanka SL, Kälber T, Soliva CR, Kreuzer M, Zeitz JO 2012. Effect of feeding dried high-sugar ryegrass ('AberMagic') on methane and urinary nitrogen emissions of primiparous cows. Livestock Science 150(1): 293-301.

Statistics New Zealand 2015. Economic indicators. Retrieved February 2017 http://www.stats.govt.nz/infoshare/

Steg A, van Straalen WM, Hindle VA, Wensink WA, Dooper FMH, Schils RLM 1994. Rumen degradation and intestinal digestion of grass and clover at two maturity levels during the season in dairy cows. Grass and Forage Science 49(4): 378-390.

Stern WR, Donald CM 1962. Light relationships in grass-clover swards. Crop and Pasture Science 13(4): 599-614.

Stewart A, Hayes R 2011. Ryegrass breeding-balancing trait priorities. Irish Journal of Agricultural and Food Research 50(1): 31-46.

Stewart A, Kerr G, Lissaman W, Rowarth J 2014. Grasses. In: Davis K, Casey M ed. Pasture and Forage Plants for New Zealand. Dunedin, New Zealand, New Zealand Grassland Association.

Sun XZ, Waghorn GC 2012. Improving *in sacco* incubation technique to evaluate fresh forage for selecting fast-degrading perennial ryegrass (*Lolium perenne* L.). Grass and Forage Science 67(3): 437-445.

Sun XZ, Waghorn GC, Clark H 2010. Cultivar and age of regrowth effects on physical, chemical and *in sacco* degradation kinetics of vegetative perennial ryegrass (*Lolium perenne* L.). Animal Feed Science and Technology 155(2): 172-185.

Sun XZ, Waghorn GC, Hatier JB, Easton HS 2012. Genotypic variation in *in sacco* dry matter degradation kinetics in perennial ryegrass (*Lolium perenne* L.). Animal Production Science 52(7): 566-571.

Tamminga S 1996. A review on environmental impacts of nutritional strategies in ruminants. Journal of Animal Science 74(12): 3112-3124.

Tas BM, Taweel HZ, Smit H, Elgersma A, Dijkstra J, Tamminga S 2005. Effects of perennial ryegrass cultivars on intake, digestibility, and milk yield in dairy cows. Journal of Dairy Science 88(9): 3240-3248.

Tas BM, Taweel HZ, Smit HJ, Elgersma A, Dijkstra J, Tamminga S 2006a. Effects of perennial ryegrass cultivars on milk yield and nitrogen utilization in grazing dairy cows. Journal of Dairy Science 89(9): 3494-3500.

Tas BM, Taweel HZ, Smit HJ, Elgersma A, Dijkstra J, Tamminga S 2006b. Rumen degradation characteristics of perennial ryegrass cultivars during the growing season. Animal Feed Science and Technology 131(1): 103-120.

Tavendale D, Pacheco MH, Lane GA, Fraser K, Death AF, Burke JL, Hichey MJ, Cosgrove GP 2006. The effects of ryegrass varieties differing in soluble sugar content on the rumen fermentation of amino acids and consequences for milk flavour chemistry. Proceedings of the New Zealand Grassland Association 68: 261-265.

Taweel HZ, Tas BM, Smit HJ, Elgersma A, Dijkstra J, Tamminga S 2005a. Effects of feeding perennial ryegrass with an elevated concentration of water-soluble carbohydrates on intake, rumen function and performance of dairy cows. Animal Feed Science and Technology 121(3): 243-256.

Taweel HZ, Tas BM, Smit HJ, Elgersma A, Dijkstra J, Tamminga S 2005b. Improving the quality of perennial ryegrass (*Lolium perenne* L.) for dairy cows by selecting for fast clearing and/or degradable neutral detergent fiber. Livestock Production Science 96(2): 239-248.

Thompson D 2013. Yield and nutritive value of irrigated tall fescue compared with orchardgrass: In monocultures or mixed with alfalfa. Canadian Journal of Plant Science 93(5): 799-807.

Thornton RF, Minson DJ 1972. The relationship between voluntary intake and mean apparent retention time in the rumen. Crop and Pasture Science 23(5): 871-877.

Totty VK, Greenwood SL, Bryant RH, Edwards GR 2013. Nitrogen partitioning and milk production of dairy cows grazing simple and diverse pastures. Journal of Dairy Science 96(1): 141-149.

Turner LB, Humphreys MO, Cairns AJ, Pollock CJ 2002. Carbon assimilation and partitioning into non-structural carbohydrate in contrasting varieties of *Lolium perenne*. Journal of Plant Physiology 159(3): 257-263.

Turner LR, Donaghy DJ, Lane PA, Rawnsley RP 2006. Effect of defoliation management, based on leaf stage, on perennial ryegrass (*Lolium perenne* L.), prairie grass (*Bromus willdenowii* Kunth.) and cocksfoot (*Dactylis glomerata* L.) under dryland conditions. 1. Regrowth, tillering and water‐soluble carbohydrate concentration. Grass and Forage Science 61(2): 164-174.

Turner LR, Donaghy DJ, Pembleton KG, Rawnsley RP 2015. Longer defoliation interval ensures expression of the 'high sugar' trait in perennial ryegrass cultivars in cool temperate Tasmania, Australia. Journal of Agricultural Science 153(6): 995-1005.

van Dorland HA, Wettstein HR, Leuenberger H, Kreuzer M 2006. Comparison of fresh and ensiled white and red clover added to ryegrass on energy and protein utilization of lactating cows. Animal Science 82(5): 691-700.

van Soest PJ, Robertson JB, Lewis BA 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. Journal of Dairy Science 74(10): 3583-3597.

van Vuuren AM, Tamminga S, Ketelaar RS 1991. *In sacco* degradation of organic matter and crude protein of fresh grass (*Lolium perenne*) in the rumen of grazing dairy cows. Journal of Agricultural Science 116(3): 429-436.

Verbič J, Ørskov ER, Žgajnar J, Chen X, Žnidaršič-Pongrac V 1999. The effect of method of forage preservation on the protein degradability and microbial protein synthesis in the rumen. Animal Feed Science and Technology 82(3): 195-212.

Vibart RE, Tavendale MH, Burke JL, Pacheco D 2012. *In vitro* fermentation of [^{15}N-] ryegrass and ruminal digesta of sheep grazing a ryegrass-based pasture in the morning or in the afternoon. Proceedings of the New Zealand Society of Animal Production 72: 100-105.

Wade MH, Peyraud JL, Lemaire G, Comeron EA 1989. The dynamics of daily area and depth of grazing and herbage intake of cows in a five day paddock system. 16th International Grassland Congress. Pp. 1111-1112.

Waite R, Boyd J 1953. The water-soluble carbohydrates of grasses. I.Changes occurring during the normal life-cycle. Journal of the Science of Food and Agriculture 4(4): 197-204.

Weller RF, Cooper A 2001. Seasonal changes in the crude protein concentration of mixed swards of white clover/perennial ryegrass grown without fertilizer N in an organic farming system in the United Kingdom. Grass and Forage Science 56(1): 92-95.

Wilkins PW 1991. Breeding perennial ryegrass for agriculture. Euphytica 52(3): 201-214.

Wilkins PW, Humphreys MO 2003. Progress in breeding perennial forage grasses for temperate agriculture. Journal of Agricultural Science 140(2): 129-150.

Wilkins PW, Allen DK, Mytton LR 2000. Differences in the nitrogen use efficiency of perennial ryegrass varieties under simulated rotational grazing and their effects on nitrogen recovery and herbage nitrogen content. Grass and Forage Science 55(1): 69-76.

Williams YJ, Doyle PT, Egan AR, Stockdale CR 2005a. Increasing the intake of highly digestible Persian clover herbage reduces rumen fluid pH and the rate of degradation of neutral detergent fibre in grazing dairy cows. Animal Production Science 45(12): 1529-1537.

Williams YJ, Walker GP, Doyle PT, Egan AR, Stockdale CR 2005b. Rumen fermentation characteristics of dairy cows grazing different allowances of Persian clover- or perennial ryegrass-dominant swards in spring. Animal Production Science 45(6): 665-675.

Wilman D, Asiegbu JE 1982. The effects of clover variety, cutting interval and nitrogen application on herbage yields, proportions and heights in perennial ryegrass-white clover swards. Grass and Forage Science 37(1): 1-13.

Wilman D, Mtengeti EJ, Moseley G 1996. Physical structure of twelve forage species in relation to rate of intake by sheep. Journal of Agricultural Science 126(3): 277-285.

Wilson D 1995. Endophyte: the evolution of a term, and clarification of its use and definition. Oikos 73(2): 274-276.

Wims CM, Ludemann CI, Phillips H, Chapman DF 2017. The economic value to dairy systems of genetic gains in the nutritive value of perennial ryegrass in grass-clover pastures. Animal Production Science 57(7): 1357-1365.

Wims CM, McEvoy M, Delaby L, Boland TM, O'Donovan M 2013. Effect of perennial ryegrass (*Lolium perenne* L.) cultivars on the milk yield of grazing dairy cows. Animal 7(3): 410-421.

Woodfield DR, Easton HS 2004. Advances in pasture plant breeding for animal productivity and health. New Zealand Veterinary Journal 52(6): 300-310.

Wu Z, Kanneganti VR, Massingill LJ, Wiltbank MC, Walgenbach RP, Satter LD 2001. Milk production of fall-calving dairy cows during summer grazing of grass or grass-clover pasture. Journal of Dairy Science 84(5): 1166-1173.

Xie W, Robins JG, Escribano S, Bushman BS, Zhang X 2014. Cultivar × binary mixture interaction effect on agronomic traits in orchardgrass. Grassland Science 60(2): 104-111.

Yamada T, Jones ES, Cogan NOI, Vecchies AC, Nomura T, Hisano H, Shimamoto Y, Smith KF, Hayward MD, Forster JW 2004. QTL analysis of morphological, developmental, and winter hardiness-associated traits in perennial ryegrass. Crop Science 44(3): 925-935.

Zemenchik RA, Albrecht KA, Shaver RD 2002. Improved nutritive value of Kura clover- and birdsfoot trefoil-grass mixtures compared with grass monocultures. Agronomy Journal 94(5): 1131-1138.

Refereed Publications during the course of study

Chen A, Bryant RH, Edwards GR 2016. Dietary preference of dairy cows for perennial ryegrass cultivars growing with and without white clover. *Proceedings of the New Zealand Society of Animal Production* 76: 81-86. (Chapter 6)

Chen A, Bryant RH, Edwards GR 2017. Milk production and composition of dairy cows grazing two perennial ryegrass cultivars allocated in the morning and afternoon. *Animal Production Science* 57(7): 1507-1511. (Chapter 7)

Dietary preference of dairy cows for perennial ryegrass cultivars growing with and without white clover

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Abstract

Dietary preference of dairy cows for eight perennial ryegrass cultivars (AberMagic, Alto, Base, Bealey, Commando, Kamo, One50 and Prospect) growing with and without white clover was examined at two vegetative stages (May and October) and one reproductive stage (November). Groups of dairy cows (n = 8) were offered free choice among cultivars growing with or without white clover for 6 to 8 hours. Preference was defined as the relative decreasing rate in sward surface height. Preference was higher for the tetraploid cultivars, Base and Bealey and the high-sugar diploid cultivar, AberMagic (preference ranged from 1.02 to 1.45). Preference was negatively correlated with herbage mass (r = -0.179, P = 0.013), proportion of dead material (r = -0.301, P < 0.001) and neutral detergent fibre (r = -0.287, P < 0.001), and positively correlated with sward surface height (r = 0.386, P < 0.001), ryegrass lamina length (r = 0.233, P = 0.001), tiller mass (r = 0.338, P < 0.001), water-soluble carbohydrate concentration (r = 0.143, P = 0.049) and organic matter digestibility in dry matter (r = 0.312, P < 0.001). Although the proportion of white clover was low (< 7% DM) in all three experiments, the interactions between perennial ryegrass cultivar and the presence of white clover were significant (P = 0.004 at pre-heading stage, P = 0.046 at reproductive stage and P = 0.038 at post-heading stage), with differences among preference for perennial ryegrass cultivars reduced when white clover was present.

Keywords: dietary preference; perennial ryegrass; white clover; interaction

Introduction

An understanding of the dietary preference of grazing animals is important for the efficient utilization of pasture (Rutter et al. 2004). It is also useful for both farmers and breeders to improve animal voluntary intake and performance (Edwards et al. 2008). Preference is exhibited not only for different species (Francis et al. 2006; van Dorland et al. 2006), but also for cultivars of the same species (Shewmaker et al. 2006; Solomon et al. 2014). Previous studies indicated that preference was related to pre-grazing herbage mass, leaf proportion (Solomon et al. 2014) and herbage nutritive value (Smit et al. 2006).

Dietary preference of dairy cows, beef cattle and sheep has been evaluated for perennial ryegrass (*Lolium perenne*) cultivars (Roegiers et al. 1988; Smit et al. 2006). Most of these studies have been carried out using pure perennial ryegrass pastures rather than perennial ryegrass/white clover mixtures. Sowing white clover (*Trifolium repens*) with perennial ryegrass is common in temperate pastures (Peoples & Baldock 2001). The interaction between pasture components can diminish or enhance the differences among perennial ryegrass cultivars (Lee et al. 2012). A small change in white clover percentage could lead to a disproportionate effect on animal production and outweigh the cultivar effect of perennial ryegrass (Hyslop et al. 2000). Hypothetically, including white clover might lead to a change of dietary preference for perennial ryegrass cultivars.

The objectives of this study were (1) to measure the sward structure, morphology, chemical composition and digestibility of herbage, and dietary preference of dairy cows, for eight perennial ryegrass cultivars growing with and without white clover, and (2) to define what herbage characteristics contributed to preference.

Materials and methods

Experimental design

Three grazing preference experiments were conducted when perennial ryegrass reached three growth stages: vegetative, post-heading (autumn 2013); vegetative, pre-heading (early spring 2014); and reproductive (late spring 2014). The pasture was established in March 2012 in a split-plot design with four blocks, with the main-plot factor being white clover (absence or presence) and the sub-plot factor being perennial ryegrass cultivar. A main-plot with an area of 18 m × 28.8 m was fenced using electric wires, resulting in eight paddocks (two levels of the main-factor × four blocks). Each main-plot contained eight adjacent sub-plots (18 m × 3.6 m), consisting of randomly allocated perennial ryegrass cultivars (two tetraploids, Base and Bealey, sown at 28 kg/ha; six diploids, AberMagic, Alto, Commando, Kamo, One50 and Prospect, sown at 20 kg/ha). The white clover used in the experiment was a mixture of cultivar Kopu II and Tribute (sown at 4 kg/ha). Sowing rates of diploid and tetraploid cultivars were designed to generate similar plant populations at establishment. To remove potential bias from previous grazing, management of treatment plots prior to the experiments were standardised by mowing all plots to achieve similar residual heights.

Animal management

In each grazing experiment, sixty-four Friesian × Jersey crossbred dairy cows were randomly divided into eight groups. Each group of eight cows was allocated to one of the main-plots, each containing eight adjacent perennial ryegrass cultivars after morning milking (around 0730). Cows grazed the plots with free access to all eight cultivars for six to eight hours depending on the herbage availability. Cows had *ad libitum* access to water.

Sward measurements

Herbage mass was measured prior to grazing by cutting three 0.2 m^2 quadrats in each sub-plot to ground level with hand shears. A subsample of 50 g fresh weight was taken from each quadrat for botanical composition and morphological analysis. Perennial ryegrass, white clover (in treatments where white clover was present), dead material and weeds were separated by hand. Ten intact perennial ryegrass tillers were selected randomly and the length of the newest fully expanded leaf blades (from ligule to tip) were recorded. All samples were oven-dried at 60°C for 48 h and weighed so the botanic composition could be calculated.

In order to monitor the decrease in sward height during grazing, 30 measurements of perennial ryegrass sward surface height (SSH, excluding headings) were recorded in each sub-plot at 0, 1, 2, 4 and 6 or 8 h after the start of grazing using a sward stick (Jenquip, New Zealand). The height of white clover petiole was recorded using a sward stick before grazing.

Chemical composition and digestibility analysis

Additional herbage samples of 150 g fresh weight were cut to ground level in each sub-plot before grazing. Samples were oven-dried at 60°C for 48 h, weighed and ground through a 1-mm sieve. Ground samples were analysed by NIRS (near infrared reflectance spectroscopy systems 5000, Foss, Maryland, USA) for chemical composition, including the concentrations of organic matter (OM), crude protein (CP), acid detergent fibre (ADF), neutral detergent fibre (NDF) and water-soluble carbohydrates (WSC) and organic matter digestibility in dry matter (DOMD).

Preference calculation

In this study, preference was defined as the decreasing rate of SSH at the beginning of grazing when herbage availability was ample and constraints associated with diet choices were minimal (Hodgson 1979). As the dietary preference for a food choice can be affected by other choices that the animals have at the same time, preference should be a value relative to the average of all the choices (Hodgson 1979).

A growth model was used to fit the decline in perennial ryegrass SSH at different times during grazing:

$$y = a(1 - e^{-bx})$$

Consequently, the preference, α was calculated as follows:

$$\alpha_i = \frac{y'_{(x=0)_i}}{\sum_j^8 y'_{(x=0)_j}/8}, \qquad j = 1, 2, \cdots 7, 8$$

where y is the accumulative decline in SSH (cm), x is the time after the start of grazing (h), a is the predicted final decline of SSH (cm), b is the fractional decreasing rate of sward height and $y'_{(x=0)i}$ is the SSH decreasing rate at the beginning of grazing ($x = 0$) of cultivar i.

At the beginning of grazing, among all the options the cows had, the faster the SSH decreased, the greater the preference. A preference of 1.00 would be representative of a cultivar if it is exactly intermediate, neither being preferred nor non-preferred.

Statistical analysis

The effects of growing with or without white clover (WC, main-factor), perennial ryegrass cultivar (PRG, sub-factor) and their interactions (WC × PRG) on sward structure, morphology characteristics, chemical composition, digestibility and preference were analysed by ANOVA (SPSS version 22.0) for the each of the three growth stages using a split-plot model. Pearson correlation analysis was performed between the preference and pasture measurements, which were converted to relative values according to the mean within a paddock to enable the comparison among paddocks and growth stages.

Results

Sward structure and morphology

Inclusion of white clover had negligible impact on the sward structure and morphology characteristics (Table 1). The tetraploid cultivars Base and Bealey had lower herbage

Table 1 Sward structure and morphology characteristics of perennial ryegrass pastures growing with and without white clover. P values for main-effect of white clover are shown

	With	Without	S.E.M.	P value
Pre-heading stage				
Sward surface height (cm)	25.2	25.1	0.4	0.903
Herbage mass (DM kg/ha)	3234	3460	166	0.406
Perennial ryegrass (%)	75.4	81.0	0.6	0.008
White clover (%)	6.4	-	-	-
Weeds (%)	1.5	1.8	0.5	0.703
Dead material (%)	16.2	16.8	0.6	0.472
Ryegrass lamina length (cm)	13.1	13.5	0.1	0.055
White clover petiole length (cm)	11.1	-	-	-
Tiller mass (mg)	46.0	56.2	2.0	0.036
Reproductive stage				
Sward surface height (cm)	23.6	24.1	0.4	0.528
Herbage mass (DM kg/ha)	3593	3438	200	0.584
Perennial ryegrass (%)	77.3	80.5	0.8	0.077
White clover (%)	6.8	-	-	-
Weeds (%)	1.4	1.6	0.2	0.435
Dead material (%)	14.5	16.4	0.8	0.114
Ryegrass lamina length (cm)	12.0	13.1	0.5	0.212
White clover petiole length (cm)	12.0	-	-	-
Tiller mass (mg)	55.8	56.7	1.5	0.679
Post-heading stage				
Sward surface height (cm)	18.4	17.5	0.1	0.023
Herbage mass (DM kg/ha)	1370	1399	95	0.846
Perennial ryegrass (%)	71.0	72.8	0.9	0.254
White clover (%)	4.9	-	-	-
Weeds (%)	1.0	0.8	0.3	0.748
Dead material (%)	23.1	26.3	1.1	0.142
Ryegrass lamina length (cm)	15.1	14.0	0.3	0.109
White clover petiole length (cm)	10.2	-	-	-
Tiller mass (mg)	27.2	25.7	0.5	0.127

Table 2 Sward structure and morphology characteristics of eight perennial ryegrass cultivars. P values for sub-effect of perennial ryegrass are shown. Means within rows followed by different subscripts are significantly different according to Duncan (P < 0.05)

	Base	Bealey	AberMagic	Alto	Commando	Kamo	One50	Prospect	S.E.M.	P value
Pre-heading stage										
Sward surface height (cm)	23.1^{d}	22.6^{d}	23.2^{d}	25.0^{c}	28.6^{a}	28.9^{a}	22.7^{d}	26.9^{b}	0.5	<0.001
Herbage mass (DM kg/ha)	2690^{d}	2652^{d}	3194^{c}	3580^{bc}	4135^{a}	3958^{ab}	3320^{c}	3249^{c}	155	<0.001
Perennial ryegrass (%)	78.4	79.2	79.4	77.6	78.8	76.1	79.3	76.8	1.0	0.178
White clover (%)	5.7	8.2	7.4	4.6	6.1	7.2	4.7	7.6	1.9	0.790
Weeds (%)	2.4^{ab}	2.9^{a}	1.9^{abc}	2.0^{abc}	0.9^{c}	1.1^{bc}	1.1^{bc}	1.3^{bc}	0.4	0.021
Dead material (%)	16.2^{bcd}	13.7^{d}	14.4^{cd}	16.4^{bc}	17.2^{ab}	19.1^{a}	17.2^{ab}	17.7^{ab}	0.8	0.001
Ryegrass lamina length (cm)	14.2^{a}	13.8^{ab}	11.6^{c}	14.0^{ab}	13.7^{ab}	13.2^{ab}	12.9^{abc}	12.8^{bc}	0.4	0.002
White clover petiole length (cm)	9.1^{c}	9.5^{c}	11.7^{ab}	11.9^{a}	12.6^{a}	12.7^{a}	10.0^{bc}	11.7^{ab}	0.6	0.001
Tiller mass (mg)	55.9^{b}	67.5^{a}	44.3^{d}	55.7^{b}	47.5^{cd}	38.6^{e}	52.4^{bc}	47.0^{cd}	2.0	<0.001
Reproductive stage										
Sward surface height (cm)	23.6^{b}	25.4^{a}	24.6^{ab}	24.6^{ab}	23.3^{b}	20.7^{c}	23.2^{b}	25.4^{a}	0.5	<0.001
Herbage mass (DM kg/ha)	2948^{d}	2990^{d}	3551^{bc}	3912^{ab}	3755^{ab}	3253^{cd}	4039^{a}	3675^{ab}	148	<0.001
Perennial ryegrass (%)	81.9	78.4	78.9	77.2	79.7	76.2	78.4	80.2	1.3	0.015
White clover (%)	4.2	10.7	10.1	4.8	4.1	9.9	3.7	7.1	2.1	0.079
Weeds (%)	1.3^{b}	2.8^{a}	1.1^{b}	2.0^{ab}	1.0^{b}	1.1^{b}	1.5^{b}	0.9^{b}	0.4	0.015
Dead material (%)	14.0^{bc}	12.3^{c}	14.1^{bc}	17.4^{a}	16.8^{ab}	16.8^{ab}	17.1^{a}	15.1^{ab}	0.9	<0.001
Ryegrass lamina length (cm)	12.0^{abc}	13.3^{ab}	11.8^{bc}	13.6^{ab}	12.6^{ab}	10.6^{c}	12.5^{ab}	13.7^{a}	0.6	0.006
White clover petiole length (cm)	10.8^{c}	10.6^{bc}	12.5^{ab}	11.5^{abc}	12.1^{abc}	12.8^{a}	12.5^{ab}	12.9^{a}	0.4	0.040
Tiller mass (mg)	66.4^{a}	74.0^{a}	53.1^{bc}	55.^{7bc}	47.3^{cd}	39.9^{d}	57.6^{b}	55.8^{bc}	2.8	<0.001
Post-heading stage										
Sward surface height (cm)	17.1^{bc}	18.6^{a}	18.7^{a}	18.1^{ab}	17.7^{ab}	16.2^{c}	18.6^{a}	18.7^{a}	0.4	0.001
Herbage mass (DM kg/ha)	1088^{b}	1335^{a}	1540^{a}	1308^{ab}	1495^{a}	1413^{a}	1476^{a}	1445^{a}	78	0.011
Perennial ryegrass (%)	75.0^{a}	74.8^{a}	71.7^{ab}	70.5^{b}	69.3^{b}	73.1^{ab}	70.4^{b}	69.8^{b}	1.2	0.004
White clover (%)	5.2	6.7	6.3	3.3	3.4	5.1	3.5	5.6	1.7	0.701
Weeds (%)	0.8	1.5	1.2	0.8	0.9	0.7	0.7	0.9	0.3	0.621
Dead material (%)	21.6^{c}	20.4^{c}	24.0^{abc}	27.0^{ab}	28.4^{a}	23.6^{bc}	26.9^{ab}	26.5^{ab}	1.4	0.002
Ryegrass lamina length (cm)	14.7^{abc}	16.1^{a}	14.3^{bc}	14.4^{abc}	13.2^{cd}	12.3^{d}	16.0^{a}	15.5^{ab}	0.5	<0.001
White clover petiole length (cm)	8.9^{bc}	8.1^{c}	10.9^{ab}	10.5^{ab}	10.4^{ab}	10.6^{ab}	10.6^{ab}	11.8^{a}	0.7	0.027
Tiller mass (mg)	24.7^{b}	37.9^{a}	26.1^{b}	25.5^{b}	23.1^{bc}	20.1^{c}	26.7^{b}	27.0^{b}	1.4	<0.001

mass and heaver tillers than other cultivars (Table 2). There was no difference in the proportion of white clover in perennial ryegrass cultivars at all three growth stages. There were no interactions between white clover and perennial ryegrass cultivar for sward structure and morphology.

Chemical composition and digestibility

Overall herbage CP concentration was higher and ADF and NDF concentrations were lower when white clover was included (Table 3). Tetraploid cultivars, Base and Bealey, had lower ADF and NDF concentrations than other cultivars at all three stages (Table 4). Generally, AberMagic had the highest WSC concentration, while Alto, Commando and Kamo were low in DOMD.

Preference

Table 5 shows relative preference of each cultivar against all cultivars. Across all three experiments, the tetraploid cultivars, Base and Bealey, and the high-sugar diploid cultivar AberMagic, were more preferred cultivars (preference ranged from 1.02 to 1.45). There were significant interactions (P < 0.05), indicating that the dietary preference for different perennial ryegrass cultivars was affected by the presence of white clover. With few exceptions (such as One50 at pre-heading stage), almost all preferences gathered towards the mean, 1.00, when white clover was present in the pasture.

Correlations

Preference was negatively correlated with herbage mass (r = -0.179, P = 0.013), dead material proportion (r = -0.301, P < 0.001), ADF (r = -0.336, P < 0.001) and NDF (r = -0.287, P < 0.001) concentration and positively correlated with SSH (r = 0.386, P < 0.001), the proportion of perennial ryegrass (r = 0.223, P = 0.002), lamina length (r = 0.233, P = 0.001), tiller mass of ryegrass (r = 0.338, P < 0.001), OM (r = 0.206, P = 0.004) and WSC (r = 0.143, P = 0.049) concentration and DOMD (r = 0.312, P < 0.001). White clover and weed proportion, white clover petiole length and CP concentration were not correlated with preference (P > 0.05).

Discussion

Sward structure, morphology, chemical composition and digestibility

There was a marked effect of perennial ryegrass cultivar on sward structure, morphology, chemical composition and

Table 3 Herbage organic matter (OM, mg/g), crude protein (CP, mg/g), acid detergent fibre (ADF, mg/g), neutral detergent fibre (NDF, mg/g) and water-soluble carbohydrates (WSC, mg/g) and organic matter digestibility in dry matter (DOMD, %) of perennial ryegrass pastures growing with and without white clover. P values for main-effect of white clover are shown.

	With	Without	S.E.M.	P value
Pre-heading stage				
OM	903	907	1	0.079
CP	171	154	2	0.007
ADF	243	248	1	0.033
NDF	470	486	3	0.023
WSC	215	233	1	0.001
DOMD	73.6	73.6	0.2	0.940
Reproductive stage				
OM	903	904	3	0.857
CP	165	145	4	0.082
ADF	267	279	2	0.036
NDF	513	540	4	0.045
WSC	187	195	11	0.702
DOMD	68.6	67.0	0.6	0.200
Post-heading stage				
OM	868	864	2	0.234
CP	249	230	4	0.049
ADF	268	281	3	0.038
NDF	493	521	5	0.023
WSC	63	60	2	0.459
DOMD	67.4	65.5	0.6	0.116

digestibility. In keeping with previous studies (Roegiers et al. 1988; Smith et al. 2001), the tetraploid cultivars, Base and Bealey, had lower SSH and herbage mass and fewer and heavier tillers. Further, consistent with previous studies (Moorby et al. 2006; Wims et al. 2013), the tetraploid cultivars and the high-sugar diploid cultivar, AberMagic, had a greater digestibility and lower fibre content.

Growing white clover in mixed pastures had negligible impact on perennial ryegrass sward structure and morphology, although it did affect some aspects of herbage chemical composition, such as CP, ADF and NDF concentrations. This was most likely due to the greater CP concentration and lower ADF and NDF contents of white clover than perennial ryegrass (Evans et al. 1996). However, the overall size of white clover effect was small compared to that associated with cultivar. For example, ADF concentration decreased from 248 to 243 mg/g when white clover was included, while ADF concentration ranged from 234 to 261 mg/g among perennial ryegrass cultivars at post-heading stage. The possible reason for the limited impact on perennial ryegrass sward structure and morphology could be the low white clover proportion in mixture pastures (< 7% DM), which was, in turn, caused by the high annual nitrogen application rate (325 kg N/ha) suppressing the growth of white clover (Labuschagne et al. 2006).

It was also noteworthy that there were limited effects of perennial ryegrass cultivar on white clover content, with no significant difference in the proportion of white clover

Table 4 Herbage organic matter (OM, mg/g), crude protein (CP, mg/g), acid detergent fibre (ADF, mg/g), neutral detergent fibre (NDF, mg/g) and water-soluble carbohydrates (WSC, mg/g) and organic matter digestibility in dry matter (DOMD, %) of eight perennial ryegrass cultivars. P values for sub-effect of perennial ryegrass are shown. Means within rows followed by different subscripts are significantly different according to Duncan (P < 0.05).

	Base	Bealey	AberMagic	Alto	Commando	Kamo	One50	Prospect	S.E.M.	P value
Pre-heading stage										
OM	904^{bc}	905^{bc}	912^{a}	903^{bc}	908^{ab}	900^{c}	907^{ab}	904^{bc}	2	0.006
CP	166	174	163	160	158	161	154	164	5	0.140
ADF	245^{c}	234^{d}	234^{d}	252^{b}	254^{b}	261^{a}	241^{c}	243^{c}	2	<0.001
NDF	472^{c}	456^{d}	464^{cd}	488^{b}	499^{a}	507^{a}	468^{c}	473^{c}	3	<0.001
WSC	217^{bc}	224^{b}	254^{a}	214^{bc}	224^{b}	196^{c}	241^{ab}	221^{bc}	8	0.001
DOMD	74.0^{cd}	75.2^{ab}	75.9^{a}	73.0^{de}	72.1^{e}	70.3^{f}	74.6^{bc}	73.7^{cd}	0.4	<0.001
Reproductive stage										
OM	910^{a}	905^{ab}	910^{a}	900^{bc}	897^{c}	894^{c}	904^{ab}	906^{ab}	2	<0.001
CP	143^{b}	164^{a}	156^{ab}	152^{ab}	163^{a}	163^{a}	145^{b}	149^{ab}	5	0.001
ADF	270^{bcd}	257^{d}	262^{cd}	276^{abc}	289^{a}	287^{ab}	275^{bc}	272^{c}	4	<0.001
NDF	522^{cd}	500^{d}	509^{cd}	531^{bc}	555^{a}	547^{ab}	530^{bc}	525^{bc}	7	0.001
WSC	220^{a}	208^{ab}	219^{a}	184^{b}	150^{c}	145^{c}	200^{ab}	198^{ab}	8	<0.001
DOMD	69.4^{abc}	70.0^{ab}	70.6^{a}	67.4^{c}	64.4^{d}	64.7^{d}	67.3^{c}	68.0^{bc}	0.7	<0.001
Post-heading stage										
OM	868^{abc}	868^{ab}	870^{a}	863^{bc}	862^{c}	869^{a}	865^{abc}	862^{c}	2	0.010
CP	252^{a}	254^{a}	227^{c}	227^{c}	231^{c}	245^{ab}	234^{bc}	245^{ab}	4	<0.001
ADF	273^{bc}	265^{d}	270^{cd}	282^{a}	281^{a}	272^{c}	278^{ab}	274^{bc}	2	<0.001
NDF	503^{cd}	489^{e}	499^{de}	519^{ab}	522^{a}	505^{cd}	515^{abc}	509^{bcd}	4	<0.001
WSC	59^{bc}	71^{b}	86^{a}	60^{bc}	47^{c}	$58b^{c}$	61^{bc}	48^{c}	4	<0.001
DOMD	67.4^{a}	68.4^{a}	67.9^{a}	65.1^{c}	64.5^{c}	66.9^{ab}	65.7^{bc}	65.5^{bc}	0.5	<0.001

Table 5 Effect of white clover on the preference of dairy cows for eight perennial ryegrass cultivars. Means within rows followed by different subscripts are significantly different according to Duncan ($P < 0.05$). Preference has been defined as the decreasing rate in sward surface height relative to the average across all cultivars. Greater preference means more preferred; a preference of 1.00 means intermediate.

White clover	Perennial ryegrass cultivar									P value		
	Base	Bealey	AberMagic	Alto	Commando	Kamo	One50	Prospect	S.E.M.	WC	PRG	PRG × WC
Pre-heading stage												
Without	1.29	1.24	1.36	0.74	0.89	0.72	0.76	1.00				
With	1.05	1.04	1.31	1.00	1.03	1.09	0.63	0.85				
Mean	1.17^{ab}	1.14^{b}	1.34^{a}	0.87^{c}	0.96^{c}	0.91^{c}	0.70^{d}	0.92^{c}	0.09	-	<0.001	0.004
Reproductive stage												
Without	1.09	1.67	1.14	0.89	0.83	0.38	0.71	1.31				
With	1.06	1.23	1.25	0.85	0.95	0.72	0.93	1.01				
Mean	1.07^{bcd}	1.45^{a}	1.19^{ab}	0.87^{d}	0.89^{cd}	0.55^{e}	0.82^{d}	1.16^{bc}	0.13	-	<0.001	0.046
Post-heading stage												
Without	1.14	1.34	1.09	1.05	0.95	0.61	0.64	1.18				
With	0.89	1.26	0.99	0.86	1.12	0.79	1.16	0.94				
Mean	1.02^{b}	1.30^{a}	1.04^{ab}	0.95^{bc}	1.04^{ab}	0.70^{c}	0.90^{bc}	1.06^{ab}	0.12	-	0.003	0.038

among perennial ryegrass cultivars at all three experiments. Frame & Boyd (1986) hypothesised that perennial ryegrass cultivars with an open structure and a lower tiller density, such as tetraploid cultivars, could support more white clover than those diploid cultivars with a dense sward. However, that was not confirmed in this study, perhaps due to low proportions of white clover in the pasture (< 7% DM).

Preference

This study examined the effect of inclusion of white clover in a pasture sward on preference for perennial ryegrass cultivars, as measured by decline in SSH during short-term grazings. A feature of the results was that the differences among dietary preference for perennial ryegrass cultivars were reduced when white clover was present. This effect was most noticeable at the reproductive stage in late spring when the quality of the pasture was the lowest. Given the low overall proportion of white clover in pasture, the results of reduced preference is surprising. The reduced preference probably reflects the partial preference that has been previously shown for white clover (Francis et al. 2006; Rutter et al. 2004), diminishing the effect of perennial ryegrass cultivar *per se*. This is supported by Cosgrove et al. (2002) who showed that dietary preference between ryegrass with a high and low nitrogen concentration was reduced when white clover was offered as another choice.

There was a consistent effect of perennial ryegrass cultivar on preference of dairy cows, such that tetraploid cultivars (Base and Bealey) and high sugar diploid cultivar (AberMagic) were the most preferred in all three experiments. This finding agrees with previous studies (Roegiers et al. 1988; Smit et al. 2006). Previous studies suggested that taller swards are preferred by livestock (Illius et al. 1992). Although, there was a positive correlation between SSH and preference across cultivars, the tetraploid cultivars were not always taller than the diploid counterparts; thus preference for tall herbage is unlikely to explain the strong preference shown for the tetraploid cultivars. In agreement with previous studies

Table 6 Pearson correlation coefficients between preference of dairy cows for perennial ryegrass cultivars and sward structure, morphology characteristics, chemical components and digestibility of perennial ryegrass pastures (n=190).

	Correlation coefficient	P value
Sward surface height	0.386	<0.001
Herbage mass	-0.179	0.013
Perennial ryegrass proportion	0.223	0.002
White clover proportion†	0.038	0.714
Weed proportion	-0.008	0.913
Dead material proportion	-0.301	<0.001
Lamina length	0.233	0.001
Petiole length†	-0.069	0.507
Tiller mass	0.338	<0.001
OM	0.206	0.004
CP	0.070	0.337
ADF	-0.336	<0.001
NDF	-0.287	<0.001
WSC	0.143	0.049
DOMD	0.312	<0.001

†n=95 for white clover measurements

(Mayland et al. 2000; Smit et al. 2006), correlation analyses in this study showed positive correlations between preference and digestibility and WSC concentration, and negative concentrations between preference and ADF and NDFconcentrations. Tetraploid cultivars were characterised as having high WSC concentration and digestibility, and low fibre content compared to other cultivars and these factors may have contributed to the high overall preference for these cultivars.

Conclusion

The tetraploid perennial ryegrass cultivars, Base and Bealey, and high-sugar diploid cultivar, AberMagic were preferred by dairy cows. Although white clover had limited effect on sward structure and morphology of perennial ryegrass pastures, its presence reduced the differences of dietary preference for perennial ryegrass cultivars. These findings may have implications for evaluation of perennial ryegrass cultivars for livestock production, although grazing efficiency and milk production studies are required to confirm this.

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References

Cosgrove G, Anderson CB, Parsons AJ, Brock JL, Tilbrook JC 2002. Can nitrogen-fertilised ryegrass substitute for white clover? Proceedings of the New Zealand Grassland Association, West Coast, New Zealand 2002. Pg. 205-210.

Edwards GR, Parsons AJ, Bryant RH 2008. Manipulating dietary preference to improve animal performance. Animal Production Science 48: 773-779.

Evans DR, Humphreys MO, Williams TA 1996. Forage yield and quality interactions between white clover and contrasting ryegrass varieties in grazed swards. The Journal of Agricultural Science 126: 295-299.

Frame J, Boyd AG 1986. Effect of cultivar and seed rate of perennial ryegrass and strategic fertilizer nitrogen on the productivity of grass/white clover swards. Grass and Forage Science 41: 359-366.

Francis SA, Chapman DF, Doyle PT, Leury BJ 2006. Dietary preferences of cows offered choices between white clover and 'high sugar' and 'typical' perennial ryegrass cultivars. Animal Production Science 46: 1579-1587.

Hodgson J 1979. Nomenclature and definitions in grazing studies. Grass and Forage Science 34: 11-17.

Hyslop MG, Fraser TJ, Smith DR, Knight TL, Slay MWA, Moffat CA, Peterson SW 2000. Liveweight gain of young sheep grazing tall fescue or perennial ryegrass swards of different white clover content. Proceedings of the New Zealand Society of Animal Production, Hamilton, New Zealand 2000. Pg. 51-54.

Illius AW, Clark DA, Hodgson J 1992. Discrimination and patch choice by sheep grazing grass-clover swards. Journal of Animal Ecology: 183-194.

Labuschagne J, Hardy MB, Agenbag GA 2006. The effects of strategic nitrogen fertiliser application during the cool season on perennial ryegrass-white clover pastures in the Western Cape Province 3. Clover content. South African Journal of Plant and Soil 23: 269-276.

Lee JM, Matthew C, Thom ER, Chapman DF 2012. Perennial ryegrass breeding in New Zealand: a dairy industry perspective. Crop and Pasture Science 63: 107-127.

Mayland HF, Shewmaker GE, Harrison PA, Chatterton NJ 2000. Nonstructural carbohydrates in tall fescue cultivars: Relationship to animal preference. Agronomy journal 92: 1203-1206.

Moorby JM, Evans RT, Scollan ND, MacRae JC, Theodorou MK 2006. Increased concentration of water-soluble carbohydrate in perennial ryegrass (*Lolium perenne* L.). Evaluation in dairy cows in early lactation. Grass and Forage Science 61: 52-59.

Peoples MB, Baldock JA 2001. Nitrogen dynamics of pastures: nitrogen fixation inputs, the impact of legumes on soil nitrogen fertility, and the contributions of fixed nitrogen to Australian farming systems. Animal Production Science 41: 327-346.

Roegiers P, Reheul D, van Bogaert G 1988. The persistence of tetraploid perennial ryegrass in a mixture with diploid perennial ryegrass. Journal of Agronomy and Crop Science 161: 40-44.

Rutter SM, Orr RJ, Yarrow NH, Champion RA 2004. Dietary preference of dairy cows grazing ryegrass and white clover. Journal of Dairy Science 87: 1317-1324.

Shewmaker GE, Mayland HF, Roberts CA, Harrison PA, Chatterton NJ, Sleper DA 2006. Daily carbohydrate accumulation in eight tall fescue cultivars. Grass and Forage Science 61: 413-421.

Smit HJ, Tamminga S, Elgersma A 2006. Dairy cattle grazing preference among six cultivars of perennial ryegrass. Agronomy journal 98: 1213-1220.

Smith KF, Simpson RJ, Culvenor RA, Humphreys Mervyn O, Prud'Homme MP, Oram RN 2001. The effects of ploidy and a phenotype conferring a high water-soluble carbohydrate concentration on carbohydrate accumulation, nutritive value and morphology of perennial ryegrass (*Lolium perenne* L.). The Journal of Agricultural Science 136: 65-74.

Solomon JKQ, Macoon B, Lang DJ, Vann RC, Ward S 2014. Cattle grazing preference among tetraploid and diploid annual ryegrass cultivars. Crop Science 54: 430-438.

van Dorland HA, Wettstein HR, Leuenberger H, Kreuzer M 2006. Comparison of fresh and ensiled white and red clover added to ryegrass on energy and protein utilization of lactating cows. Animal Science 82: 691-700.

Wims CM, McEvoy M, Delaby L, Boland TM, O'Donovan M 2013. Effect of perennial ryegrass (*Lolium perenne* L.) cultivars on the milk yield of grazing dairy cows. Animal 7: 410-421.

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Milk production and composition of dairy cows grazing two perennial ryegrass cultivars allocated in the morning and afternoon

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Abstract. The objective of the study was to evaluate the effect of perennial ryegrass cultivar and timing of herbage allocation on herbage nutritive value and milk production of mid-lactation dairy cows. An autumn grazing trial using 48 Friesian × Jersey spring-calving cows was conducted over 10 days. Twelve groups of four cows were allocated to three replicates of four treatments, namely, two perennial ryegrass cultivars (AberMagic or Prospect) offered either after milking in the morning (0830 hours) or afternoon (1630 hours). Cows were offered a daily herbage allowance of 30 kg DM/cow above ground level. There were no significant differences in sward structure and morphological characteristics between cultivars, except for Prospect having a lower average tiller mass (43.1 mg) than AberMagic (48.4 mg). The concentration of water-soluble carbohydrates (WSC) and organic matter digestibility in DM (DOMD) was greater in AberMagic (180 g/kg, 74.2%) than in Prospect (153 g/kg, 71.4%). Herbage DM percentage, WSC concentration and DOMD were lower in the morning than in the afternoon (18.8% vs 22.3% DM; 154 vs 179 g/kg WSC; 72.1% vs 73.5% DOMD). Herbage DM intake (12.0 kg/cow.day), milk yield (17.2 kg/cow.day) and milksolids yield (1.60 kg/cow.day) did not differ significantly among treatments. Cultivar choice and timing of allocation influenced herbage WSC concentration and digestibility, but did not alter milksolids production.

Additional keywords: AberMagic, herbage allocation time, high-sugar cultivar, Prospect, water-soluble carbohydrates.

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Introduction

Pasture is a key component to sustaining dairy production in grazing systems. Perennial ryegrass (*Lolium perenne* L.) is one of the most widely used grass species, especially in temperate regions, due to its high nutritive value and annual herbage production. A range of commercial perennial ryegrass cultivars has been developed on the basis of agronomic characteristics, such as yield, nutritive value and persistence (Lee *et al*. 2012). However, less information is available of how cultivars affect feeding value and animal performance (Wims *et al*. 2013), due to the cost of comparison. In recent years, new cultivars with an elevated concentration of water-soluble carbohydrates (WSC) have been produced for greater milk production and nitrogen-use efficiency (Miller *et al*. 2001; Lee *et al*. 2012).

A further factor that may affect animal performance is the timing of herbage allocation. Herbage DM percentage and the concentration of WSC increase during the day, because of moisture loss and net photosynthesis accumulation (Orr *et al*. 2001). Accordingly, other fractions in DM, such as crude protein (CP), neutral detergent fibre (NDF) and acid detergent fibre (ADF) are diluted (Delagarde *et al*. 2000). Improved herbage digestibility has been recorded in the afternoon (Huntington and Burns 2007; Brito *et al*. 2008). Under grazing conditions, ruminants show a regular grazing pattern, with the longest and most intense grazing event taking place in the afternoon (including dusk; Gregorini 2012). Therefore, matching this grazing pattern with diurnal fluctuations in herbage nutritive value by allocating new pastures in the afternoon might enhance the performance of livestock (Orr *et al*. 2001; Gregorini *et al*. 2006). It may also improve microbial synthesis efficiency and nitrogen-use efficiency of dairy cows (Vibart *et al*. 2012), as afternoon herbage contains more WSC and less CP, providing more rapidly fermentable carbohydrates (Hristov *et al*. 2005) and producing less ammonia in the rumen (Tamminga 1996).

The objective of the present study was to investigate the effect of perennial ryegrass cultivar and timing of herbage allocation on herbage characteristics and milk production of mid-lactation cows. A high-sugar diploid cultivar, AberMagic, and the standard counterpart, Prospect, were evaluated in the study. They were similar in morphology and sward structure (Rossi *et al*. 2014), while distinct in the nutritive value, especially in the concentration of WSC that showed a greater diurnal variation (Delagarde *et al*. 2000). Interactions between cultivar and timing of allocation for herbage nutritive value and milk production were also investigated.

Materials and methods

Experimental site and design

The experiment was conducted in irrigated pastures at the Lincoln University Research Dairy Farm, Canterbury, New Zealand (43°38′S, 172°27′E, 12 m above sea level). The experiment was a 2 × 2 randomised block design with four treatments (AberMagic or Prospect allocated in the morning or afternoon). Perennial ryegrass cultivars AberMagic and Prospect were sown in adjacent strips (from 30 to 50 m in width) at 20 kg/ha without white clover in May 2015. Pastures were grazed rotationally from August 2015 to January 2016 before the experiment.

Animal management

Forty-eight Friesian × Jersey dairy cows were blocked into 12 groups with four cows each, according to milksolids yield (1.56 ± 0.01 kg/cow.day), age (6.67 ± 0.12 years), days in milk (174 ± 4.5 days) and liveweight (529.6 ± 6.1 kg). The 12 groups were assigned randomly to three replicates of four treatments in a 10-day grazing experiment (4-day transition period and 6-day experimental period). Morning and afternoon allocation groups were offered their daily fresh allowances of pasture after morning milking (0830 hours) and afternoon milking (1630 hours) respectively. Daily herbage allowance was 30 kg DM/cow above ground level for each group. Cows had *ad libitum* access to water.

Pasture measurements

To estimate herbage mass, a minimum of 30 pre- and 30 post-grazing sward compressed height measurements were taken with a rising plate meter (RPM, Jenquip, Feilding, New Zealand) in each grazing area on a daily basis. The RPM measurements (0.5-cm units) were calibrated by measuring the compressed height of herbage within a 0.2-m^2 quadrat and then harvesting this herbage to ground level. Herbage samples were oven-dried at 60°C for 48 h and weighed. Linear regression was performed between RPM and herbage mass (kg DM/ha), as follows:

$$Herbage\ mass_{pre} = 99.8 \times RPM + 1139, n = 191,$$
$$R^2 = 0.53, P < 0.01,$$
$$Herbage\ mass_{post} = 147.1 \times RPM + 331, n = 177,$$
$$R^2 = 0.48, P < 0.01.$$

Apparent herbage DM intake was calculated according to the difference between the values of pre- and post-grazing herbage mass, the area allocated and the number of cows grazed.

Herbage samples of ~500 g fresh weight were cut to ground level from at least 10 points in each group 1 h before herbage allocation. A subsample of ~50 g was weighed and oven-dried at 60°C for 48 h to obtain DM percentage. Another subsample was taken for botanical composition and morphological analysis. The sample was sorted by hand into perennial ryegrass, dead material and weeds. Ten intact perennial ryegrass tillers were selected randomly, and the lengths of the newest fully expanded leaf blades (from ligule to tip) were recorded. All samples were oven-dried at 60°C for 48 h and weighed. Botanic composition and tiller mass could be further calculated. The remaining sample was frozen immediately, freeze-dried and ground through a 1-mm sieve for nutritive value evaluation by near infrared reflectance spectroscopy (NIRS Systems 5000, Foss, MD, USA) to determine the concentrations of organic matter (OM), CP, NDF and WSC and organic-matter digestibility in DM (DOMD).

Milk measurements

Milk yield was measured daily for individual cows with an automated milking system (Alpro Herd management system, Tumba, Sweden). Milk samples were collected daily from all cows during morning and afternoon milkings throughout the experimental period, to determine milk composition and milk urea nitrogen (MUN). The samples for milk composition were analysed by the laboratory of Livestock Improvement Corporation Ltd (Christchurch, New Zealand) to determine milk fat, protein and lactose by MilkoScan (Foss Electric, Hillerod, Denmark). Samples for MUN measurement were centrifuged at 4000*g* for 10 min to allow milk fats to solidify on the top and be removed. The skim milk was pipetted into a clean micro-centrifuge tube and frozen at −20°C before analysis. Skim milk was analysed by Daytona analyser (Randox Laboratories, Crumlin, UK).

Statistical analyses

Means were calculated for each group, using data from individual measurements collected from each day and animal. All analyses were performed in SPSS version 22.0 (IBM, New York, NY, USA). Sward structure and morphological characteristics were analysed by one-way ANOVA using the following model:

$$y_{ij} = \mu + \alpha_i + \gamma_j + \varepsilon_{ij},$$

where y is the dependent variable, μ is the overall mean, α_i is the effect of cultivar (i = 1 to 2), γ_j is the block effect (j = 1 to 3) and ε_{ij} is the residual error.

Herbage chemical composition, digestibility, DM intake, milk yield and milk composition were analysed by two-way ANOVA using the following model:

$$y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \gamma_k + \varepsilon_{ijk},$$

where y is the dependent variable, μ is the overall mean, α is the effect of cultivar (i = 1–2), β is the effect of timing of allocation (j = 1–2), $(\alpha\beta)_{i,j}$ is the interaction, γ_k is the block effect (k = 1–3) and ε_{ijk} is the residual error.

Results

There were no significant differences in herbage mass and botanical composition between the cultivars. However, AberMagic tiller was 5.3 mg heavier than Prospect tiller (Table 1, $P < 0.05$). Herbage DM percentage ($P < 0.01$), WSC concentration ($P < 0.05$) and DOMD ($P < 0.01$) were all greater in the afternoon (Table 2). Although WSC concentration was greater in AberMagic than Prospect ($P < 0.05$), there was a tendency of an interaction between cultivar and timing of allocation (P = 0.07, s.e.m. = 9.1). The concentration of WSC increased more during the day in Prospect than in AberMagic (from 131 to 176 g/kg in Prospect; from 178 to 183 g/kg in AberMagic). DOMD and WSC concentration showed similar responses to the effect of perennial ryegrass cultivar and time of day. AberMagic and afternoon herbage had a greater DOMD than did Prospect ($P < 0.01$) and morning herbage ($P = 0.01$) respectively. Herbage DM percentage did not differ between the cultivars, while OM percentage, CP and NDF concentrations remained similar under different treatments (Table 2).

Apparent herbage DM intake, milk yield, milksolids yield and milk composition were unaffected by perennial ryegrass cultivar or timing of allocation (Table 3, $P > 0.05$). A significant interaction in MUN concentration ($P = 0.05$, s.e.m. = 0.21) indicated that the effect of allocation timing on MUN concentration was different between perennial ryegrass cultivars. MUN concentration decreased from 9.33 to 8.39 mmol/L when Prospect was allocated in the afternoon, while it was similar whether AberMagic was offered in the morning or afternoon (8.95 vs 8.96 mmol/L).

Table 1. Sward structure and morphological characteristics of perennial ryegrass cultivars AberMagic and Prospect

s.e.m., standard error of the mean

Parameter	AberMagic	Prospect	s.e.m.	*P*-value
Herbage mass (kg DM/ha)	2816	2708	22.6	0.08
Perennial ryegrass (%)	87.2	86.0	0.78	0.40
Weeds (%)	2.5	2.3	0.29	0.71
Dead material (%)	10.3	11.7	0.56	0.23
Tiller density (m^{-2})	5197	5336	41.0	0.14
Tiller weight (mg DM)	48.4	43.1	0.87	0.05
Leaf length (cm)	18.0	19.0	0.27	0.12

Discussion

Sward structure and nutritive value

Few studies comparing dairy cow performance on different perennial ryegrass cultivars have been able to separate the effect of morphological attributes from that of herbage chemical composition. In the current study, two cultivars, AberMagic and Prospect, were similar in sward structure and morphology. Similar physical attributes for AberMagic and Prospect were also observed by Rossi *et al.* (2014) who compared eight perennial ryegrass cultivars, including AberMagic and Prospect, and classified them as the same functional group characterised by a high tiller density and fine leaf material. In terms of chemical composition, AberMagic had a greater WSC concentration than Prospect did in the present study, which is consistent with the results of previous studies (Staerfl *et al.* 2012; Cosgrove *et al.* 2014).

There was a pronounced effect of time of day on herbage chemical composition and digestibility. In keeping with previous research (Delagarde *et al.* 2000; Gregorini *et al.* 2006; Pulido *et al.* 2015), DM percentage and WSC concentration were greater in the afternoon than in the morning, due to the water loss via transpiration and carbohydrate accumulation via photosynthesis. Also in agreement with previous studies (Brito *et al.* 2008; Huntington and Burns 2007), the enhanced WSC concentration improved herbage digestibility in the afternoon. Delagarde *et al.* (2000) noted that the amounts of CP and NDF (absolute mass) were similar in the morning and evening and their concentrations were passively diluted by the increasing

Table 2. Chemical composition and digestibility of perennial ryegrass cultivars allocated for grazing in the morning or afternoon

OM, organic matter; CP, crude protein; WSC, water-soluble carbohydrates; NDF, neutral detergent fibre; DOMD, organic matter digestibility in DM; s.e.m., standard error of the mean

Parameter	Cultivar (C)		Timing of allocation (T)		s.e.m.	*P*-value		
	AberMagic	Prospect	Morning	Afternoon		C	T	C × T
DM (%)	21.0	20.1	18.8	22.3	0.41	0.16	<0.01	0.62
OM (g/kg)	801	843	821	823	20.8	0.21	0.97	0.18
CP (g/kg)	152	157	158	151	6.3	0.57	0.47	0.16
WSC (g/kg)	180	153	154	179	6.4	0.02	0.04	0.07
NDF (g/kg)	444	476	466	453	14.1	0.16	0.54	0.18
DOMD (%)	74.2	71.4	72.1	73.5	0.26	<0.01	0.01	0.09

Table 3. DM intake, milk yield and milk composition of dairy cows grazing perennial ryegrass cultivars allocated in the morning or afternoon

s.e.m., standard error of the mean

Parameter	Cultivar (C)		Timing of allocation (T)		s.e.m.	*P*-value		
	AberMagic	Prospect	Morning	Afternoon		C	T	C × T
DM Intake (kg/cow.day)	12.1	12.0	12.4	11.7	0.32	0.87	0.17	0.31
Milk yield (kg/cow.day)	17.2	17.1	17.0	17.3	0.31	0.90	0.41	0.36
Fat (%)	5.38	5.44	5.39	5.42	0.134	0.77	0.89	0.91
Protein (%)	4.08	3.94	3.97	4.06	0.053	0.10	0.25	0.54
Lactose (%)	4.95	5.02	5.00	4.96	0.032	0.19	0.44	0.45
Milksolids yield (kg/cow.day)	1.61	1.59	1.57	1.64	0.026	0.57	0.10	0.41
Milk urea nitrogen (mmol/L)	8.96	8.87	9.13	8.69	0.151	0.45	0.12	0.05

amount of WSC. However, in the present study, the increment in WSC concentration might not be great enough to significantly dilute the concentrations of CP and NDF within the herbage DM.

There was tentative evidence of an interaction between perennial ryegrass cultivar and time of day for WSC concentration, with a greater increase in WSC concentration in Prospect during the day than in AberMagic. However, the greater initial WSC concentration in AberMagic in the morning led to a greater overall WSC concentration in AberMagic than in Prospect. It is unknown why a greater increase in WSC concentration occurred in Prospect. However, it is possible that this was caused by the greater net photosynthesis rate in Prospect and the feedback inhibition of photosynthesis due to WSC accumulation in AberMagic (Rogers *et al*. 1998).

Intake and milk production

There were negligible effects of perennial ryegrass cultivar on milk production or composition. Wims *et al*. (2013) noted that cows grazing on AberMagic recorded lower milk yield and milksolids yield than cows grazing on Bealey, a tetraploid cultivar. Compared with standard diploid cultivars, Miller *et al*. (2001) reported that the high-sugar cultivar increased milk yield. However, Cosgrove *et al*. (2007) and Moorby *et al*. (2006) found little differences in milk production between the high-sugar and standard perennial ryegrass cultivars. This lack of effect on milk production observed in the current study reflects that the differences in DM intake between cultivars were not significant or that the differences in WSC concentration were not large enough to alter animal performance (Francis *et al*. 2006; Edwards *et al*. 2007).

Offering new herbage allowance in the afternoon was expected to lead to a greater DM intake because the longest and most intense grazing events occur in the afternoon and near dusk (Orr *et al*. 1997; Gregorini 2012). However, DM intake and, thus, milksolids yield were found to be similar in the morning and afternoon allocation systems. These findings were supported by previous studies suggesting that similar intakes in the morning and afternoon allocation systems led to the similar milk production (Orr *et al*. 2001; Abrahamse *et al*. 2009; Pulido *et al*. 2015).

Milk urea nitrogen

Milk urea nitrogen was measured in the present study as an indicator of nitrogen-use efficiency (Kauffman and St-Pierre 2001). There was an interaction between cultivar and timing of allocation for MUN concentration. MUN concentration was lower when Prospect was allocated in the afternoon, indicating a better nitrogen-use efficiency, while MUN concentration remained similar regardless of when AberMagic was allocated. Edwards *et al*. (2007) noted that herbage WSC : CP ratio is related to nitrogen-use efficiency of dairy cows. In the present study, there was a greater increase in WSC concentration from morning to afternoon in Prospect than in AberMagic, while CP concentration did not change significantly between cultivars or timing of allocation. However, compared with Prospect, AberMagic did not lead to a lower MUN concentration even with a greater WSC concentration. Therefore, this indicates other factors such as nitrogen fractionation and digestibility, could contribute to nitrogen-use efficiency (Bryant *et al*. 2012; Cosgrove *et al*. 2007).

Conclusions

AberMagic and afternoon-allocated herbage had a greater WSC concentration and digestibility. However, there was little effect of these on milk production of mid-lactation dairy cows, due to no change in DM intake. The interaction observed for MUN concentration between cultivar and timing of allocation indicated that choice of cultivar and timing of herbage allocation could potentially increase nitrogen-use efficiency of dairy cows.

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References

Abrahamse P, Tamminge S, Dijkstra J (2009) Effect of daily movement of dairy cattle to fresh grass in morning or afternoon on intake, grazing behaviour, rumen fermentation and milk production. *The Journal of Agricultural Science* **147**, 721–730. doi:10.1017/S0021859609990153

Brito A, Tremblay G, Bertrand A, Castonguay Y, Bélanger G, Michaud R, Lapierre H, Benchaar C, Petit H, Ouellet D (2008) Alfalfa cut at sundown and harvested as baleage improves milk yield of late-lactation dairy cows. *Journal of Dairy Science* **91**, 3968–3982. doi:10.3168/jds.2008-1282

Bryant R, Gregorini P, Edwards G (2012) Effects of N fertilisation, leaf appearance and time of day on N fractionation and chemical composition of *Lolium perenne* cultivars in spring. *Animal Feed Science and Technology* **173**, 210–219. doi:10.1016/j.anifeedsci.2012.02.003

Cosgrove G, Burke J, Death A, Hickey M, Pacheco D, Lane G (2007) Ryegrasses with increased water soluble carbohydrate: evaluating the potential for grazing dairy cows in New Zealand. *Proceedings of the New Zealand Grassland Association* **69**, 179–185.

Cosgrove G, Mapp N, Taylor P, Harvey B, Knowler K (2014) The chemical composition of high-sugar and control ryegrasses in grazed pasture at different latitudes throughout New Zealand. *Proceedings of the New Zealand Grassland Association* **76**, 169–176.

Delagarde R, Peyraud J, Delaby L, Faverdin P (2000) Vertical distribution of biomass, chemical composition and pepsin–cellulase digestibility in a perennial ryegrass sward: interaction with month of year, regrowth age and time of day. *Animal Feed Science and Technology* **84**, 49–68. doi:10.1016/S0377-8401(00)00114-0

Edwards GR, Parsons A, Rasmussen S, Bryant RH (2007) High sugar ryegrasses for livestock systems in New Zealand. *Proceedings of the New Zealand Grassland Association* **69**, 161–171.

Francis S, Chapman D, Doyle P, Leury B (2006) Dietary preferences of cows offered choices between white clover and 'high sugar' and 'typical' perennial ryegrass cultivars. *Animal Production Science* **46**, 1579–1587. doi:10.1071/EA04085

Gregorini P (2012) Diurnal grazing pattern: its physiological basis and strategic management. *Animal Production Science* **52**, 416–430.

Gregorini P, Eirin M, Refi R, Ursino M, Ansin O, Gunter S (2006) Timing of herbage allocation in strip grazing: effects on grazing pattern and performance of beef heifers. *Journal of Animal Science* **84**, 1943–1950. doi:10.2527/jas.2005-537

Hristov A, Ropp J, Grandeen K, Abedi S, Etter R, Melgar A, Foley A (2005) Effect of carbohydrate source on ammonia utilization in lactating dairy cows. *Journal of Animal Science* **83**, 408–421. doi:10.2527/2005.832408x

Huntington G, Burns J (2007) Afternoon harvest increases readily fermentable carbohydrate concentration and voluntary intake of gamagrass and switchgrass baleage by beef steers. *Journal of Animal Science* **85**, 276–284. doi:10.2527/jas.2006-370

Kauffman A, St-Pierre N (2001) The relationship of milk urea nitrogen to urine nitrogen excretion in Holstein and Jersey cows. *Journal of Dairy Science* **84**, 2284–2294. doi:10.3168/jds.S0022-0302(01)74675-9

Lee J, Matthew C, Thom E, Chapman D (2012) Perennial ryegrass breeding in New Zealand: a dairy industry perspective. *Crop & Pasture Science* **63**, 107–127. doi:10.1071/CP11282

Miller L, Moorby JM, Davies DR, Humphreys MO, Scollan ND, MacRae JC, Theodorou MK (2001) Increased concentration of water-soluble carbohydrate in perennial ryegrass (*Lolium perenne* L.): milk production from late-lactation dairy cows. *Grass and Forage Science* **56**, 383–394. doi:10.1046/j.1365-2494.2001.00288.x

Moorby JM, Evans RT, Scollan ND, MacRae JC, Theodorou MK (2006) Increased concentration of water-soluble carbohydrate in perennial ryegrass (*Lolium perenne* L.). Evaluation in dairy cows in early lactation. *Grass and Forage Science* **61**, 52–59. doi:10.1111/j.1365-2494.2006.00507.x

Orr R, Penning P, Harvey A, Champion R (1997) Diurnal patterns of intake rate by sheep grazing monocultures of ryegrass or white clover. *Applied Animal Behaviour Science* **52**, 65–77. doi:10.1016/S0168-1591(96)01120-3

Orr R, Rutter S, Penning P, Rook A (2001) Matching grass supply to grazing patterns for dairy cows. *Grass and Forage Science* **56**, 352–361. doi:10.1046/j.1365-2494.2001.00284.x

Pulido RG, Ruiz-Albarrán M, Balocchi OA, Nannig P, Wittwer F (2015) Effect of timing of pasture allocation on production, behavior, rumen function, and metabolism of early lactating dairy cows during autumn. *Livestock Science* **177**, 43–51. doi:10.1016/j.livsci.2015.04.002

Rogers A, Fischer BU, Bryant J, Frehner M, Blum H, Raines CA, Long SP (1998) Acclimation of photosynthesis to elevated CO_2 under low-nitrogen nutrition is affected by the capacity for assimilate utilization. Perennial ryegrass under free-air CO_2 enrichment. *Plant Physiology* **118**, 683–689. doi:10.1104/pp.118.2.683

Rossi L, McDonagh J, McEvoy M, O'Donovan M, Lee J, Chapman D, Edwards G (2014) Implications of species and management interactions for ranking perennial ryegrass (*Lolium perenne* L.) cultivars: a progress report from two hemispheres. In 'Proceedings of the 6th Australasian dairy science symposium'. (Ed. J Roche) pp. 259–262. (Australasian Dairy Science Symposium Committee: Hamilton, New Zealand)

Staerfl S, Amelchanka S, Kälber T, Soliva C, Kreuzer M, Zeitz J (2012) Effect of feeding dried high-sugar ryegrass ('AberMagic') on methane and urinary nitrogen emissions of primiparous cows. *Livestock Science* **150**, 293–301. doi:10.1016/j.livsci.2012.09.019

Tamminga S (1996) A review on environmental impacts of nutritional strategies in ruminants. *Journal of Animal Science* **74**, 3112–3124. doi:10.2527/1996.74123112x

Vibart R, Tavendale M, Burke J, Pacheco D (2012) *In vitro* fermentation of [^{15}N-] ryegrass and ruminal digesta of sheep grazing a ryegrass-based pasture in the morning or in the afternoon. *Proceedings of the New Zealand Society of Animal Production* **72**, 100–105.

Wims C, McEvoy M, Delaby L, Boland T, O'Donovan M (2013) Effect of perennial ryegrass (*Lolium perenne* L.) cultivars on the milk yield of grazing dairy cows. *Animal* **7**, 410–421. doi:10.1017/S1751731112001814